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CARDIAC ANOXIA AS THE FACTOR DETERMINING THE OCCURRENCE OF EXPERIMENTAL VIRAL CARDITIS

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PREVIOUS reports [1] have dealt with the experimental demonstration that a variety of filtrable viruses capable of infecting the rabbit will at times produce inflammation in the heart after being inoculated at a peripheral site such as the skin and subcutaneous tissues, the testis or the upper respiratory tract. The predominant lesion is myocarditis, but not uncommonly this is accompanied with valvulitis, endocarditis or pericarditis. In those instances in which virus III or pseudorabies virus was used as the infecting agent, the lesions revealed the characteristic intranuclear inclusion bodies indicating the presence of the virus. The types of viral inflammation that are not characterized by inclusion-bearing cells can be proved to be of viral cause by the ability of a saline suspension of a heart containing such a lesion to infect another animal.

Although the occurrence and the nature of the cardiac lesion are in themselves of interest, of even greater significance is the fact that the incidence and the severity of these lesions were remarkably increased not only by the grossly crude and unphysiologic procedure of cardiac puncture but also by the intravenous injection of a solution of acacia or of an extract containing betahypophamine ("pitressin") [1a] made at the time of inoculation of virus. There are several hypotheses that might explain the mechanism by which two such physically and pharmacologically dissimilar substances bring about cardiac localization of virus. The work described here substantiates one of these—the hypothesis that cardiac anoxia is the responsible factor—and fails to support others. The experiments by which the anoxia hypothesis has been tested, the methods used in them, and their results are described in the main body of the paper. The great bulk of negative experimentation that has dis-

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1. (*a*) Pearce, J. M.: Arch. Path. **28**:827, 1939; (*b*) **34**:319, 1942. (*c*) Pearce, J. M., and Levine, H. D.: Am. Heart J. **25**:102, 1943.

proved other conceivable explanations for cardiac localization of virus under these same conditions is described in less detail in the terminal discussion.

Both substances, betahypophamine by a constriction of the coronary arteries [2] and acacia by an interference with the gaseous interchange between the hemoglobin of the red blood cells and the surrounding environment,[3] produce anoxia of the heart. If anoxia is the factor that determines the localization of virus in the heart and the consequent production of viral myocarditis or endocarditis, the administration of other drugs having an ischemic or anoxic action on the heart should result in a similar localization of peripherally inoculated virus and a corresponding inflammation of muscle, valve or pericardium. If, on the contrary, anoxia is of no localizing significance or is only one of many factors of "heart strain," "heart damage" or "overburdening" of the heart, the subjection of the experimental animal to other procedures known to affect the action of the heart or to be toxic to it or to damage the myocardium in some way other than by ischemia should bring about the localization of virus in the heart and the production there of lesions of viral origin.

Following this line of reasoning, we performed the first series of experiments described in the following pages. Rabbits inoculated peripherally with virus underwent further treatment that may be divided into two categories, the one designed to produce cardiac anoxia, and the other to affect the heart but not to cause ischemia. The preliminary localizing procedure and the incidence of cardiac lesions have then been correlated.

FIRST SERIES OF EXPERIMENTS

Method and Material.—Virus III [4] was the infecting agent used in all but a few of the experiments. This virus is highly suitable for histologic studies, since lesions caused by it constantly have in them cells containing easily recognizable large acidophilic intranuclear inclusion bodies typical of those classified by Cowdry as type A.[5] These inclusions identify the lesion, their occurrence indicating the presence of virus. It has the additional advantage that few rabbits from the available breeding stock are insusceptible to it [6] and inoculation of active virus

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3. Christie, A.; Phatak, N. M., and Olney, M. B.: Proc. Soc. Exper. Biol. & Med. **32**:670, 1935. Studdiford, W. E.: Surg., Gynec. & Obst. **64**:772, 1937. Raven, R. W.: Brit. M. J. **1**:950, 1940.

4. Rivers, T. M., and Tillett, W. S.: J. Exper. Med. **38**:673, 1923. Miller, C. P., Jr.; Andrewes, C. H., and Swift, H. F.: ibid. **40**:773, 1924. McCartney, J. E.: Annual Report of the Metropolitan Asylums Board, London, 1924-1925, p. 152.

5. Cowdry, E. V.: Arch. Path. **10**:23, 1930.

6. Pearce, J. M.: Proc. Soc. Exper. Biol. & Med. **38**:872, 1938.

can be expected to be followed almost invariably by disease. On the other hand, its virulence is so low that premature experimental deaths do not occur. In order to indicate that the phenomenon of cardiac localization is not a characteristic of virus III only, in 5 of the 88 experiments vaccine virus was used and in 6 the myxoma virus.[7]

The inoculum was a saline suspension of an infected rabbit testis that was removed aseptically from an animal killed on the fourth or fifth day after intratesticular injection of a similar virus suspension. The suspension was prepared as follows: The infected testis was ground in a mortar with sterile sand and 0.85 per cent sodium chloride solution. The resulting paste was diluted to make a 5 to 10 per cent suspension, and the supernatant fluid was used for inoculation.

All the rabbits were young males weighing between 2,000 and 3,500 Gm., the majority weighing about 2,500 Gm. No attempt was made to select them according to breed or color, however, and they were obtained from several sources.

To infect the 88 experimental animals, 0.5 to 2 cc. of virus suspension was injected into each testis. Immediately thereafter groups of 6 each were given intravenously a solution of either digitalis, papaverine hydrochloride, nikethamide, corn syrup or 0.85 per cent sodium chloride. Another group of 6 was subjected to narcosis induced by inhalation of chloroform. A group of 5 was given barium chloride intravenously, a group of 7 epinephrine hydrochloride and groups of 20 each acacia and "pitressin."

Fifty-six rabbits inoculated with virus but otherwise untreated served as controls. The results from these animals were collected during the previous studies as well as in the experiments described here.

The digitalis was administered in the form of "cedilanid"[8] in doses of 1.0 to 1.7 mg. per kilogram. To 3 of the 6 animals in this group a second dose was given four days after the first.

Papaverine hydrochloride was injected at the rate of 2 mg. per kilogram. Two animals received repeated doses. The powdered drug was dissolved in saline solution so that 1 cc. of solution contained 2 mg.

The dose of nikethamide was 45 mg. per kilogram. That this was an adequate amount to produce an effect was indicated by the fact that 2 of the animals went into convulsions and all had obvious acceleration of heart rate and respiration.

The corn syrup[9] was diluted by adding an equal amount of 0.85 per cent sodium chloride, and 50 cc. of this 50 per cent solution was injected into the marginal vein of the ear as rapidly as possible.

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7. The vaccine virus was a highly virulent strain isolated from a spontaneously infected rabbit by one of us (J. M. P.) in 1938 (J. Infect. Dis. **66**:130, 1940). The myxoma virus was procured from Dr. Richard Shope, of the Rockefeller Institute. It was the strain originally obtained from Dr. A. Moses of the Oswaldo Cruz Institute in Brazil and used by both J. R. Hobb (Am. J. Hyg. **8**:800, 1928) and T. M. Rivers (J. Exper. Med. **51**:965, 1930) and maintained by Dr. Shope in the laboratories of the Rockefeller Institute in Princeton since 1933. The strain of virus III that was used has been carried in rabbits by testicular passage in this laboratory for the past ten years. It was given to us originally by Dr. Carl TenBroeck, of the Rockefeller Institute, and was isolated originally by Dr. T. M. Rivers (J. Exper. Med. **38**:673, 1923).

8. "Cedilanid" is a brand of lanatoside C, an initial glucoside obtained from Digitalis lanata, and manufactured by the Sandoz Chemical Works, Inc., New York.

9. "Karo syrup," manufactured by Corn Products Refining Company, Argo, Ill., and Kansas City, Mo.

The chloroform was administered by inhalation from a leather cone in the open room air in quantities sufficient to produce continuous unconsciousness for sixty to one hundred minutes.

The 0.85 per cent solution of sodium chloride was injected as rapidly as the solution could be forced from a 50 cc. syringe through a no. 20 gage hollow needle into the marginal vein of the ear. A total of 100 cc. was administered.

A 1 per cent solution of barium chloride was prepared from the chemically pure reagent. Isotonic solution of sodium chloride was then added to a calculated dose of 10 mg. per kilogram, to this solution, to make a total volume of 10 cc.

The epinephrine hydrochloride was injected at the rate of 0.01 mg. per kilogram per minute during a period of two to eight minutes.

Solutions of acacia were made by dissolving the dried powder in warm 0.85 per cent saline solution in amounts sufficient to make a concentration of 20 per cent. This solution was injected intravenously as rapidly as possible in doses of approximately 50 cc.

Betahypophamine was given intravenously either in a single injection of 0.5 cc. of "pitressin" at the time of inoculation of the virus or in several doses of this drug variously spaced throughout the experimental period.

TABLE 1.—*Data on Rabbits Inoculated with Virus and Thereafter Submitted to Procedures Tending to Cause Cardiac Anoxia*

Factors Causing Myocardial Anoxia	Animals Treated	Animals with Cardiac Lesions Caused by Virus	
		Number	Percentage
Barium chloride *	5	5	100
Epinephrine hydrochloride *	7	6	85.7
Betahypophamine † *	20	10	50
Acacia *	20	18	90

* Solutions of the substances were administered by intravenous injection.
† This was administered in the form of "pitressin."

In all groups all the rabbits were killed three to six days after inoculation and autopsies made immediately. The examination always included histologic observation of the testis and the heart. Sections of tissue fixed in Zenker's solution or in a 10 per cent solution of formaldehyde were stained routinely with hematoxylin and eosin and on occasion with Giemsa stain. The hearts were examined externally in the gross but were not opened, since it had been found previously [1a] that sections more satisfactory for microscopic study could be obtained by fixing the intact organ and later trimming it and embedding it in paraffin in such a way that all four chambers and one or more valves were included in the section in their usual relationships. By cutting several parallel blocks from a single heart it was not difficult to show at least three of the valves and often four without resorting to serial section.

Results.—The four procedures described in the foregoing section which reduce the oxygen supplied to the heart were followed by a high incidence and a high degree of severity of viral lesions of the heart. As shown in table 1, carditis resulted from the intratesticular inoculation of virus when this was accompanied with one or another of these procedures, as follows: in 100 per cent of the animals given barium chloride, in 85.7 per cent of those given epinephrine hydrochloride, in

50 per cent of those given betahypophamine and in 90 per cent of those given acacia. All the other procedures were followed by an incidence and an intensity of lesions comparable to those in the controls. In no group did more than 16.7 per cent of the experimental animals show cardiac lesions, and these were all slight in extent and comparable to those seen in the 16.1 per cent of the 56 untreated animals that exhibited myocardial or endocardial viral lesions. These figures are listed in table 2.

A detailed description of the character, the localization and the severity of the lesion of the heart does not come within the scope of this paper. There are, however, two general observations that bear on the more immediate problem of the cardiac localization of peripherally introduced virus. Probably the more significant of these is the observation that the lesions which develop after acacia has been injected into

TABLE 2.—*Data on Rabbits Inoculated with Virus and Thereafter Submitted to Procedures That May Affect the Heart But Do Not Cause Myocardial Anoxia*

Factors Affecting Heart	Animals Treated	Animals with Cardiac Lesions Caused by Virus	
		Number	Percentage
100 cc. of saline solution *	6	0	0
Corn syrup *	6	1	16.7
Nikethamide *	6	0	0
Digitalis *	6	0	0
Chloroform narcosis	6	1	16.7
Papaverine hydrochloride *	6	1	16.7
Controls: virus inoculations only	56	9	16.1

* The substances were administered intravenously.

the venous blood stream are not only predominantly of the right ventricle but not uncommonly confined to the right side of the heart, while those that follow the injection of drugs that cause constriction of the coronary arteries are chiefly in the left ventricle and especially in the inner half of the myocardium and the large papillary muscles. Of less importance but certainly worthy of note in that it also supports the theory of cardiac anoxia is the observation that there is a quantitative difference in the severity of the lesions in the two large groups. Those that occurred in the small number of animals that were not submitted to procedures causing cardiac anoxia were considerably milder and less widespread than those in the anoxic group, being in general of the same order of severity as those in the nontreated controls.

COMMENT ON FIRST SERIES

The anatomic distribution of lesions between the two ventricles furnishes secondary evidence that a lack of oxygen is of importance in the development of viral lesions of the heart, since it is exactly the

distribution of the areas to which the flow of oxygen is diminished by the different actions of the drugs constricting the coronary arteries and acacia. Thus in the case of constriction of the coronary arteries the right ventricle and the auricles are spared because the muscular walls of these chambers are so thin in the rabbit and contain so many endothelium-lined crevices communicating with the cavities that they are less dependent on coronary circulation and may receive a major part of their oxygen and nutriment directly from the blood within them. The thick left ventricle, however, is supplied by the blood flowing through the coronary arteries, and when that flow is diminished the muscle suffers. The inner portions of the myocardium and the plump papillary muscles of the left ventricle receive the terminal branches of the arteries and are therefore the regions most severely affected by the narrowing of the lumens of these vessels. This narrowing obviously may be brought about by the active contraction of the smooth muscle of the arterial walls that occurs under betahypophamine [2] or barium chloride stimulation [10]; or it may result from the great increase in the intramyocardial pressure that occurs, as well as the increase in intracardiac pressure, during the period of increased contractile force and acceleration of rate [11] brought about by epinephrine. The elevation of arterial blood pressure that follows the administration of all three substances also causes the intramyocardial pressure to rise. In vivo experiments conducted in this laboratory by our colleagues Johnson and Di Palma [12] have demonstrated that during systole the intramyocardial pressure of the left ventricle exceeds the intra-aortic and hence the intracoronary pressure. The coronary arteries are thereby compressed and the volume of blood flowing through them to the inner part of the myocardium and the papillary muscles is diminished. As was indicated earlier by Anrep and his associates,[13] in strongly beating hearts the volume of blood flowing through even the more superficial arteries is reduced by the increase of intramyocardial pressure brought about by the stronger contraction.

The entirely different mechanism by which acacia produces cardiac anoxia explains why the lesions are predominantly on the right side of the heart when an intravenous injection of this substance is combined with the inoculation of virus. The acacia interferes with the interchange of oxygen between the hemoglobin of the red blood cells and the sur-

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10. Sollmann,[2c] p. 554.

11. Goodman, L., and Gilman, A.: The Pharmacological Basis of Therapeutics, New York, The Macmillan Company, 1941, p. 403.

12. Johnson, J. R., and Di Palma, J. R.: Am. J. Physiol. **125**:234, 1939.

13. Anrep, G. V.; Cruickshank, E. W. H.; Downing, A. C., and Subba Rau, A.: Heart **14**:111, 1927. Anrep, G. V., and Hausler, H.: J. Physiol. **65**:357, 1928. Anrep, G. V.; Davis, J. C., and Volhard, E.: ibid. **73**:405, 1931. Anrep, G. V., and von Saalfedd, E.: ibid. **79**:317, 1933.

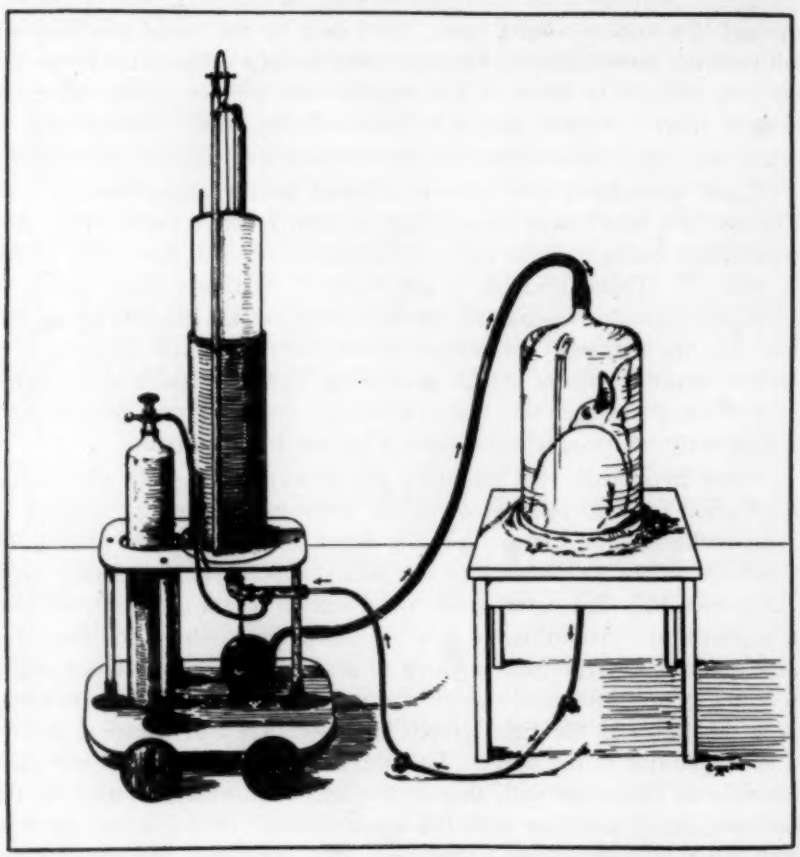
rounding fluids and tissues. This action has been well demonstrated by Christie, Phatak and Olney [8] and others and has been used to explain the cases of accidental death that followed the use of this substance as a plasma substitute some years ago. The greatest dilution of blood and hence the greatest interference with the passing of oxygen from red blood cell to tissue fluid occurs in the right auricle and ventricle as the acacia pours in from the injection site, the marginal vein of the ear, through the superior vena cava. Not only is the blood reaching the left ventricle more highly oxygenated after passing through the lungs, but also the mixture of blood of the superior and inferior venae cavae has become more complete and has decreased the local concentration of acacia and thereby diminished the interference with gaseous interchange.

Those procedures which were followed by the appearance of viral lesions of the heart have been placed in table 1, while those which only occasionally brought about cardiac localization of virus have been placed in table 2. Those in table 1 also have in common their ability to produce myocardial anoxia and therefore bear out the original hypothesis that it is the deficiency of oxygen which determines the occurrence of the viral carditis. Those in table 2, although they are capable of damaging the heart or altering its function in a variety of ways, do not tend directly or indirectly to decrease the oxygen available to that organ.

Other hypotheses that might be put forward to explain the cardiac localization of viral lesions follow the somewhat indefinite concept of "overburdening" or "straining" the heart and thereby decreasing its resistance to the localization of the small amounts of circulating virus that presumably had entered the blood stream at the site and the time of inoculation. According to this reasoning, the sudden addition of a large quantity of hypertonic solution of acacia to the venous blood might so increase its volume and thereby the venous blood pressure as to cause acute dilatation of the right auricle and ventricle and transient failure of the right side of the heart. The prevalence of the lesion of the right ventricle as compared with that of the left when acacia is used as the localizing agent is in line with this explanation. That it is not the true explanation has been shown by two types of experiments. In one of these, measurements of the blood pressure of the right carotid artery and of that of the marginal vein of the left ear were made and recorded by a kymograph before, during and after the period in which acacia or isotonic solution of sodium chloride was injected into the marginal vein of the right ear of the rabbit. With the doses used in the work tabulated here, no significant alteration occurred in either pressure. In the other type of experiment direct observations of the heart of the intact anesthetized animal were made while acacia was injected into the aural vein. No dilatation of the right ventricle could be made out. However, pallor of the right ventricle and auricle appeared almost immediately after

the injection was started and persisted until the inflow of solution of acacia was stopped.

Electrocardiograms [14] taken during the injection of acacia solution have failed to show any change in the recorded activity of the heart other than that in the rate of contraction. Slight acceleration was the rule, but occasionally slowing occurred.



Apparatus combining a bell jar sealed with "plasticine," a spirometer and a nitrogen cylinder by which rabbits were subjected to an atmosphere deficient in oxygen.

Although the interpretation of the results of these experiments on the basis of cardiac anoxia is convincing it rests to a large extent on indirect evidence. Therefore, in order to confirm it, a further series of experiments has been carried out, in which rabbits have been submitted for longer or shorter periods to an atmosphere deficient in oxygen.

14. In the obtaining of electrocardiograms and their interpretation we were aided by Dr. Harold D. Levine, of Boston.

SECOND SERIES: CARDITIS RESULTING FROM EXPERIMENTAL LOWERING OF ATMOSPHERIC OXYGEN

Method and Material.—By combining a bell jar with a spirometer an apparatus (figure) has been constructed in which rabbits can be placed in an atmosphere abnormally high in nitrogen and low in oxygen. The expired carbon dioxide and water vapor are absorbed by soda lime through which the gases circulate. Eighteen experiments were performed. In each of these an animal was inoculated intratesticularly with virus III and immediately thereafter placed in the bell jar, and the jar was flushed with nitrogen several times. The system was then closed, but at five minute intervals samples of gas were withdrawn and the concentrations of oxygen, carbon dioxide and nitrogen determined. The maximum and minimum readings of oxygen content were 12 and 4.5 per cent in the several experiments, and in each experiment the oxygen content decreased progressively from the beginning to the end of the period. When the concentration of oxygen approached 4.5 per cent, the animal became unconscious and had to be removed from the chamber. There was considerable variation among animals in the degree of oxygen deprivation that caused collapse. The time during which exposure occurred was determined by the tolerance of the animal to the anoxia and by the amount of oxygen in the chamber at the beginning of the experiment. In the 18 experiments the time of exposure ranged between six and forty-one minutes, but

TABLE 3.—*The Incidence of Cardiac Lesions in Rabbits Subjected to an Atmosphere Deficient in Oxygen*

	Animals with Cardiac Lesions Caused by Virus	
Animals Treated	Number	Percentage
18	12	66.7

in the majority it was twenty to thirty minutes. As in the preceding group of experiments, the rabbits were killed three to six days after inoculation and exposure to oxygen-deficient atmosphere. The hearts, the testes and the brains were examined for inflammation and inclusion-bearing cells.

Results.—The hearts of 12 of the 18 animals inoculated with virus III and thereafter deprived of the normal amount of atmospheric oxygen contained inflammatory lesions in which there were virus III inclusion bodies (table 3). These lesions were predominantly in the heart muscle (myocarditis), but were occasionally found in the endocardium or the epicardium. Although their incidence, 66.7 per cent, was comparable to that in the previous group of experiments in which acacia, epinephrine, barium chloride or betahypophamine ("pitressin") was used as the localizing factor the individual lesions tended to be less severe. There were no lesions in the brain.

SUMMARY AND CONCLUSIONS

It has been shown that inflammatory lesions occur on occasion in the muscle and membranes of the heart in rabbits suffering from a variety of viral infections. However, the incidence and the severity of

these lesions are markedly increased when the animals are submitted to procedures that tend to decrease the amount of oxygen supplied to the heart. The procedures that have been successful in inducing virus to localize in the heart and there cause inflammation are (1) the administration of drugs that diminish the coronary blood flow both by active constriction of the arteries—betahypophamine ("pitressin"), barium chloride, epinephrine—and by passive compression due to the contracting myocardium and (2) the administration of substances that interfere with the passing of oxygen from erythrocyte to tissue (acacia).

Finally, the fact that anoxia is the determining factor in the occurrence of this experimental viral carditis has been demonstrated in direct experiments in which anoxia was produced by placing the experimental animal in an atmosphere deficient in oxygen.

SIMULTANEOUS CHROMAFFIN TUMORS OF THE CAROTID BODY AND THE GLOMUS JUGULARIS

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SINCE the recognition of the right and left carotid bodies, there have been numerous isolated reports of tumors arising from them. The first was published by Marchand in 1891. In 1929 Bevan and McCarthy [1] collected 133 cases from the literature and added 1 of their own. In 1931 Rankin and Wellbrock [2] added 12 cases, including a case of bilateral tumor. Chase [3] in 1933 reported 2 cases from this institute. The patients were sisters, and in one of them the tumor was bilateral.

Tumor of a carotid body associated with tumor of other chromaffin tissue is rare. Gilford and Davis [4] described a carotid body tumor with a nodule in the liver resembling the tumor of the carotid body. In 1934 Clagg [5] reported tumors occurring simultaneously in a carotid body and an organ of Zuckerkandl. Goodof and Lischer [6] in 1943 recorded a carotid body tumor with a histologically similar tumor of the body of the pancreas. More recently, Rosenwasser [7] described a carotid body tumor arising in the left middle ear and mastoid process and suggested as its origin the glomus jugularis. This structure is described as being found in the apex of the bulb of the jugular vein, immediately below the bony floor of the middle ear and near the ramus tympanicus of the glossopharyngeal nerve.[8] In view of all these reports the description of another single case of carotid body tumor would seem to be redundant. However, in the case to be reported there were two chromaffin tumors, one in the right carotid body area and one in the petrous portion of the left temporal bone. Since it is the second reported case of a carotid body-like tumor arising in the temporal bone, the rarity of this site of origin and the duality make it a case of unusual interest.

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REPORT OF A CASE

A Jewish woman was first admitted to the Royal Victoria Hospital in December 1933. At that time she was 29 years of age. Her complaint concerned a discharge of the left ear which had persisted for one year prior to admission. Her own physician on two occasions had removed polyps from the left external auditory canal, but polypoid growth had recurred. When she was examined, there was no tenderness over the mastoid process, but the external auditory canal was blocked in its inner portion by a granular growth about which there was some bloody discharge. A first stage radical left mastoidectomy was performed. The polypoid growth of the external auditory canal was found to extend into the cells of the mastoid process a short distance from the middle ear. Pathologic examination of tissue fragments from the external ear revealed an appearance suggestive of angiomatous polyp. The second stage of the operation was completed nine days later and was followed by an uneventful recovery.

The patient entered another hospital on March 29, 1943. At this time she stated that in 1938 she had first noticed swelling in the right side of the neck and that this swelling had slowly enlarged. A purulent discharge of the left ear had been present since December 1942. On March 31 a biopsy specimen was removed from a polyp discovered in the left auditory meatus, and an attempt was made to remove the cervical tumor. There was severe hemorrhage before the completion of the latter operation, so that the tumor was not removed. About eight hours after operation the patient had palsy of the left side of the face, some difficulty in speaking and paralysis of the left arm and leg. On April 1 the right pupil was dilated and fixed and the left pupil small and fixed. A lumbar puncture was done several hours later, and a diagnosis of hemorrhage of the right internal capsule was made. The neoplasm in the right side of the neck was believed to be a tumor of the carotid body.

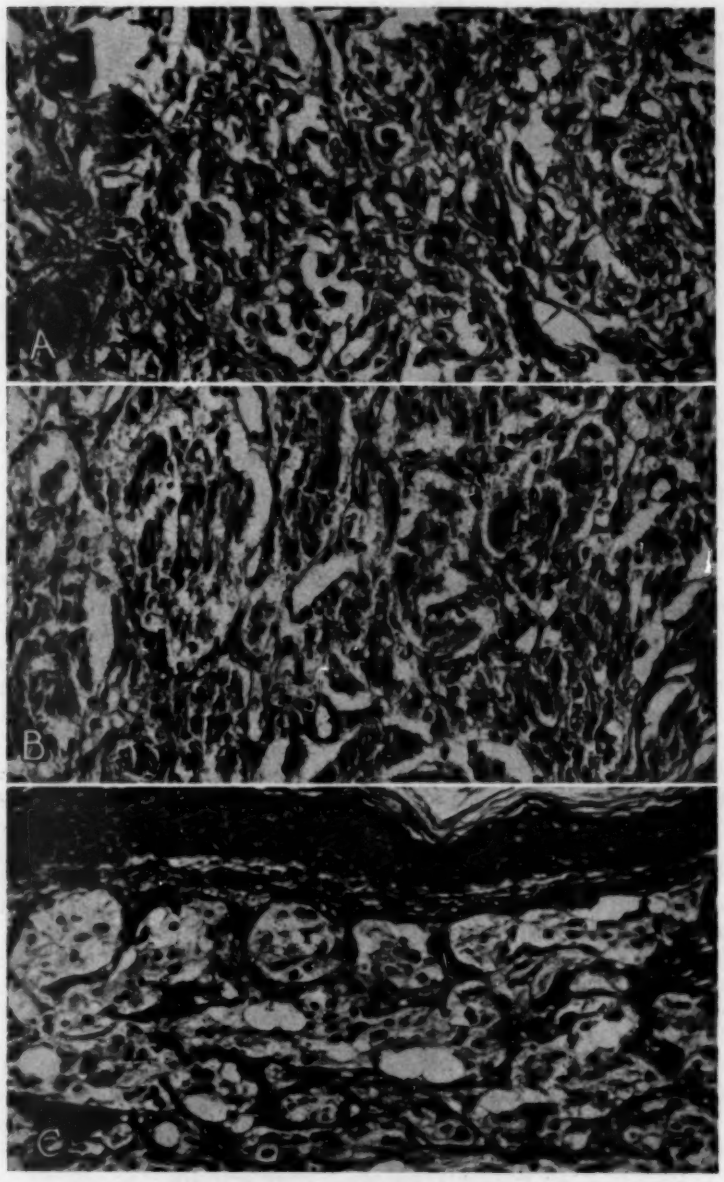
The patient was transferred on April 1 to the Montreal Neurological Institute for emergency operation. She was unconscious and on admission was taken to the operating room for ventriculography. She died before this was completed. The impression was that there was at least one and perhaps more expanding lesions in the right cerebral hemisphere. Roentgen examination disclosed that a considerable portion of the apex of the petrous portion of the left temporal bone was destroyed, probably by neoplasm.

Postmortem Examination.—A complete autopsy was performed four hours after death by Dr. H. B. Jackson. The pertinent findings were confined to the head and the neck, and only these will be described in the following summary.

A mass, which measured 2.5 cm. in diameter and extended upward for a distance of 6.5 cm., was found at the bifurcation of the right common carotid artery. It appeared to have a well defined capsule. A catgut suture completely surrounded the external carotid artery 3 cm. above the bifurcation. One centimeter above the origin of the internal carotid artery a catgut suture was found passing through the lumen of the vessel, occluding about half of its circumference. Jelly-like blood clot was found above the ligature. At the upper pole of the tumor there was a lymph node measuring 1.5 cm. in diameter.

The pyramid of the petrous portion of the left temporal bone was destroyed by a soft tissue mass, but this had not penetrated the dura. The neoplasm extended into the middle ear cavity and into the left external auditory canal. It was soft and lobulated, having the same consistency as pancreatic tissue.

In the brain, the gray and the white matter and the basal ganglions on the right side presented extensive recent softening.



A, photomicrograph of tumor of the right carotid body. The vacuolated cells present possessed finely granular eosinophilic cytoplasm. Hematoxylin-eosin stain; × 230.

B, photomicrograph of tumor of the petrous portion of the left temporal bone. The cytoplasm of the clear cells which encroach on the bony trabeculae was eosinophilic and finely granular. Hematoxylin-eosin stain; × 230.

C, photomicrograph of the original biopsy specimen from the left external auditory canal. Stratified squamous epithelium of the external auditory canal overlies nests of tumor cells immediately beneath. Hematoxylin-eosin stain; × 200.

Microscopic Examination.—After suitable fixation, blocks were cut from the tumor removed from the right side of the neck and the one from the left petrous temporal bone and stained with hematoxylin and eosin, Masson's trichrome stain, Schmorl's stain for chromaffin granules, Verhoeff's stain for elastic tissue and reticulum stains. The cervical tumor was well encapsulated and at the periphery an occasional arteriole contained recent thrombotic material. It was composed of nests and sheets of cells separated by bands of collagenous fibrous connective tissue. In most instances the nests contained six to eight cells, which tended to have a pseudoalveolar arrangement with small capillaries and sinusoids at their peripheries. A delicate fibrous network was scattered throughout the tumor. The cells of the tumor were irregularly polyhedral, although some appeared to have fine fibrils extending from them. The cytoplasm was finely granular and eosinophilic with hematoxylin and eosin. A few of the cells possessed clear, almost colorless cytoplasm. The nuclei were spherical. Many were solid and basophilic, while others were vesicular, with one to three nucleoli. An occasional cell contained two or three hyperchromatic nuclei, but no mitotic figures were discerned. With Schmorl's chromaffin stain, some of the cells were seen to contain bluish green granules, although many were clear (*A* in figure). No tumor cells were found in the lymph node removed from the upper pole of the tumor.

The same histologic picture was encountered in the tumor removed from the petrous portion of the left temporal bone, although here the general tissue structure was looser (*B* in figure). Here, too, Schmorl's stain showed some of the tumor cells to be filled with bluish green granules. In this tumor there were moderate numbers of spicules of bone. Many of these were completely viable, but others were basophilic in their staining capacities. Some had tumor cells in close contact with them, while in other areas multinucleated cells possessing four to five pale basophilic nuclei and eosinophilic cytoplasm were encountered about the necrotic bony spicules.

When the biopsy specimens taken from the left external auditory meatus in December 1933 were reviewed, two histologic pictures were apparent. The tissue fragments were covered by unremarkable stratified squamous epithelium on which there was a little lamellated keratin. Beneath this epithelium, granulation tissue containing numerous blood capillaries and supported by older, partially hyalinized fibrous connective tissue was seen. However, at the base of one of the tissue fragments a different histologic picture was encountered. Here there were a few small nests of four to eight clear cells clumped together and separated by delicate strands of fibrous connective tissue. These cells had the same histologic appearance as those in the tumors of the neck and the petrous portion of the temporal bone. Small capillaries were present at the edges of the nests. Some of the cells had pale granular eosinophilic cytoplasm when stained with hematoxylin and eosin. Their nuclei were spherical or oval. Some were solid and hyperchromatic, while many were vesicular, with nucleoli. An occasional cell contained two or three nuclei, but no mitotic figures were seen (*C* in figure). Schmorl's stain for chromaffin granules revealed a rare cell containing bluish green granules.

COMMENT

Thus, in brief, there are, in this case, a tumor of the right carotid gland and a tumor of the petrous portion of the left temporal bone. The latter would appear to have been present for at least ten years, having been incompletely removed at a previous mastoidectomy.

In view of the rarity of dual carotid body–like tumors the question is raised as to whether this was a cancer of the carotid body of the right side of the neck with metastases in the petrous portion of the left temporal bone. The histologic picture of the tumor of the carotid body was not that of a cancerous lesion. It was encapsulated, there was no invasion of the capsule and no mitotic figures were seen. Moreover, the cervical tumor would appear to have arisen five years after the discovery of a tumor of identical structure in the petrous portion of the left temporal bone and external ear. The tumor of the petrous portion of the left temporal bone, although locally invasive, destroying bone in the immediate vicinity and extending into the middle and external ears, did not appear cancerous histologically. Also, the route by which tumor cells could have reached the opposite side of the neck to produce a metastasis in the right carotid body is not clear.

It would seem more reasonable to assume that there are here two independent tumors of the chromaffin system, one arising in the right carotid body and the second in a more obscure chromaffin structure. The discovery by Guild [8] of a chromaffin structure in the temporal bone, the glomus jugularis, provides a morphologic explanation of the origin of a tumor of this type in the petrous portion of the temporal bone.

The chromaffin tumor of the petrous portion of the left temporal bone is similar in many respects to the one recently reported by Rosenwasser. Both patients when originally presenting themselves complained of a growth in the external canal of the left ear and diminishing hearing. Rosenwasser's patient disclaimed discharge of the ear until four weeks prior to admission to the hospital for mastoidectomy, while the patient in the present case had aural discharge intermittently for eleven years prior to death. The growth in the left external auditory canal in both cases had apparently been present for ten years. The original biopsy specimen from the external auditory canal was reported as "vascular granulation tissue" in Rosenwasser's case and as "angiomatous polyp" in the one presented here.

At mastoidectomy both the tumors resembled granulation tissue and bled excessively. Microscopically, the histologic picture of the tissue removed at mastoidectomy in Rosenwasser's case and the appearance of that in the base of the original biopsy specimen from the external auditory canal in 1933 in our case and of the tissue found in the petrous portion of the left temporal bone at autopsy were essentially similar. Furthermore, the same picture was thought to be present in Rosenwasser's case when the original biopsy specimen from the external auditory canal was reviewed. The tumor nests of relatively large cells in all were separated by relatively dense fibrous connective tissue and tended to be bordered by capillaries and sinusoids. In some areas the cells formed sheets and cordlike structures. Some of the cells of both cases contained

vacuolated cytoplasm, and no mitotic figures were found in any of the sections. Occasional cells in the case reported here had bluish green granules in them, although none were demonstrated in that of Rosenwasser.

SUMMARY

A case of a tumor of the carotid body of the right side of the neck associated with a histologically similar tumor of the petrous portion of the left temporal bone is presented. The case is interesting because of the rarity of multiple tumors arising in the chromaffin system and because of the possibility that the tumor of the petrous portion of the temporal bone arose from a small and only recently recognized body known as the glomus jugularis. It is also noteworthy that the tumor of the petrous portion of the left temporal bone would appear to have been present for five years before the tumor of the right side of the neck was discovered and for ten years prior to death.

PATHOGENESIS OF GLOMERULONEPHRITIS AND RHEUMATIC FEVER

In Vivo Activation of Tissue Antigens as a Result of Streptococcic Infection and Consecutive Formation of Autoantibodies

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IN PRECEDING publications it has been shown that autoantibodies to kidney,[1] heart and connective tissue [2] can be produced in rats and rabbits by immunizing the animals with emulsions of the homologous tissues in conjunction with killed streptococci. It has further been reported that in rats such autoantibodies can be pathogenic in that they react with the tissues in situ, autoantibodies to kidney thus producing glomerulonephritis [3] and autoantibodies to heart and connective tissue producing cardiac lesions.[4] It has been pointed out that these results lend support to the view that autoantibodies to certain tissue substances might play a role in the genesis of human glomerulonephritis and rheumatic fever. The first step of this pathogenetic mechanism—during the streptococcic infection preceding these diseases—would consist of the formation of an autogenous antigen, which is effected when streptococci or their products come in contact with substances of certain tissues of the host, such as kidney, heart or connective tissue, the streptococcic and tissue material combining in such a way that the product acts as an antigen. In response to this autoantigen, autoantibodies would be formed, and these autoantibodies in turn would react with the corresponding tissues in situ, thus producing glomerulonephritis or rheumatic fever.

In the experiments described so far,[5] the streptococcus-tissue antigens—corresponding to the hypothetic autoantigen in man—were obtained in vitro by mixing killed streptococci with emulsions of the tissues. The question arose whether such autogenous tissue antigens can

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From the George Williams Hooper Foundation for Medical Research and the Division of Medicine of the University of California Medical School.

1. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945; **40**:158, 1945.
2. Cavelti, P. A.: Arch. Path., to be published.
3. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **40**:163, 1945.
4. Cavelti, P. A.: Arch. Path., to be published.
5. Footnotes 1 to 4.

also arise in animals through the effect of streptococcic substances on the tissues in vivo. It is the purpose of the present paper to describe the experiments carried out with regard to this problem.

The general procedure of these experiments was to establish focal streptococcic infections or, in some cases, to inject large amounts of killed streptococci into rats and to investigate by serologic tests (*a*) whether a tissue antigen could be demonstrated in the serum of these rats by the use of a potent antiserum to rat tissues and (*b*) whether subsequently autoantibodies were formed, demonstrable by the use of rat tissue extracts as test antigens.

MATERIALS AND METHODS

Rats of the Evans strain were used throughout (rats of the Curtis-Dunning strain were not available at the time of these studies).

The streptococci used most extensively were strain Dochez N. Y. 5 and its animal passage product E14. Two other stains of group A streptococci, derived from local human sources (nos. 17 and G22), were also employed in some of the rats.

The organisms were administered in the following preparations:

1. The bacterial sediment of 5 cc. of a twenty-four hour culture in beef heart broth was resuspended in a small amount of distilled water and mixed with 3 parts of warm agar. While still fluid, 1 cc. of the mixture was injected subcutaneously into each rat.

2. The organisms were grown for twenty-four hours on beef heart broth containing ground meat (beef heart). Meat pieces 3 to 6 mm. in diameter were implanted subcutaneously in the region of the chest under aseptic conditions, the incision being closed by suture and sealed with paraffin.

3. The organisms were grown on blood agar plates for twenty-four hours. Pieces of agar about 6 mm. in diameter were then similarly implanted under the skin.

4. The organisms were grown for twenty-four hours on a pure soybean broth in the presence of chopped pieces of soybeans. A piece of soybean was then implanted subcutaneously.

5. The organisms were grown for twenty-four hours on a medium consisting of 1 part defibrinated rat blood and 2 parts isotonic solution of sodium chloride and 1 per cent dextrose. From 0.5 to 1 cc. of this culture was injected subcutaneously.

6. Organisms were grown on Bernheimer's synthetic medium [6] and lyophil-dried in the living state. These organisms were mixed with mineral oil and injected subcutaneously in quantities of 2 to 3 mg. of dried streptococci per rat.

7. Organisms grown on Bernheimer's synthetic medium were precipitated (killed) with cold acetone, washed repeatedly with acetone and dried. They were resuspended in saline solution and injected subcutaneously and intraperitoneally in quantities of 40 to 100 mg. of dry organisms per rat.

These materials were administered in one injection or one implantation.

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6. Bernheimer, A. W.; Gillman, W.; Hottle, G. A., and Pappenheimer, A. M., Jr.: J. Bact. **43**:495, 1942.

Immediately before each injection or implantation and at various intervals thereafter the rats were bled from the tail (0.5 to 1 cc. of blood being withdrawn).

Rat kidney, heart and connective tissue perfused free of blood were obtained, and 20 per cent emulsions of these tissues were prepared and preserved as described previously.[5] For serologic tests extracts of the frozen emulsions were made by centrifugation. The collodion particle technic was used exclusively for the in vitro tests as described in detail elsewhere.[7]

Antiserums to rat kidney, heart and connective tissue were prepared by immunizing rabbits with the tissue emulsions. A total of 20 or more intraperitoneal injections were given to each rabbit at intervals of three to five days, the single dose being 5 cc. of the 20 per cent emulsions or the equivalents of emulsions made from lyophil-dried tissues. Blood was obtained from the rabbits on the seventh day after the last injection. The serum was filtered through a Seitz filter and merthiolate was added, 1 : 10,000, for preservation.

RESULTS

In most of the rats the site of the focal infection showed marked inflammatory swelling during the first few days, which then gradually diminished and disappeared within seven to fourteen days. In a few rats an abscess perforated through the skin and discharged.

Serum samples taken from the rats twelve and thirty-six hours (or twenty-four and forty-eight hours in other experiments) after the initiation of the focal infection were tested for the presence of autogenous tissue antigens with serums from rabbits immunized against rat kidney, heart and connective tissue. Of each of the several serums from rabbits immunized against the same tissue, the one showing the strongest in vitro reaction was chosen. By far the most potent was the antikidney serum, which gave a 4 plus reaction in a dilution of antigen of at least 1 : 320 (the extract of 20 per cent emulsion of kidney taken as undiluted), while the antiserum to rat heart and that to connective tissue showed 2 plus reactions with antigen dilutions of 1 : 80 and 1 : 40, respectively. When the titers of these antiserums were expressed in terms of serum dilution, the differences did not emerge so clearly, but for the purpose of determining an antigen as intended the former point of view obviously is more pertinent.

The organ or tissue specificity of these antiserums was not very high. The antiheart and anti-connective-tissue serums showed extensive cross reactions with connective tissue and extract of heart, but they did not react at all or only slightly with extract of rat lung, liver or kidney. Attempts to differentiate between antibodies to rat heart and those to rat connective tissue by means of absorption with either one of these tissues did not succeed. The antikidney serum reacted slightly with rat liver, connective tissue and lung, somewhat more strongly with rat skeletal muscle and strongly with rat heart. When absorbed with sedi-

7. Cavelti, P. A.: (*a*) J. Immunol. **49**:365, 1944; (*b*) to be published.

mented particles from rat heart emulsion, all reactivity except that with kidney was removed, but the reaction with kidney was appreciably impaired. None of these antiserums showed any reaction whatsoever with normal rat serum under the conditions of the test, nor did they react with suspensions or extract of streptococci or culture filtrate or beef heart broth.

In the tests for the presence of tissue antigens in rat serum during focal streptococcic infection, the rabbit anti-rat-heart and anti-rat-connective-tissue serums were used unabsorbed, and the anti-rat-kidney serum was employed unabsorbed and also after it had been absorbed with rat heart. In small tubes with a diameter of 7 mm., 0.1 cc. of a 1:10 dilution of stock collodion particle suspension was added to 0.1 cc. of undiluted rat serum, quickly followed by 0.1 cc. of the undiluted antiserum. After mixing, the tubes were incubated at room temperature for one hour, then centrifuged for three minutes at 1,400 revolutions per minute, and the results were read. After keeping the tubes in the refrigerator for six hours and recentrifuging, the results were read again.

Reactions were obtained only in serum samples taken between twelve and forty-eight hours after initiation of the infection, the maximum reaction occurring after twenty-four to thirty-six hours. Reactions considered significant (2 plus or stronger) were recorded for 21 of the total of 46 rats. Most of these reactions occurred with the antiserum to rat kidney when the latter was used unabsorbed. With the anti-rat-kidney serum that had been absorbed with rat heart and with the anti-rat-heart and anti-rat-connective-tissue serums only a few weak reactions were observed.

The results obtained indicate that during focal streptococcic infection, in these rats, one or several tissue antigens were released into the blood stream. It is, however, not possible to decide from these results what tissues yielded these substances.

Serum samples obtained from the same rats at intervals later on were tested for the presence of autoantibodies, with extract of rat kidney and rat heart as test antigens in the same manner as described in detail elsewhere.[8] To 0.5 cc. of each of progressive serum dilutions 0.2 cc. of collodion-antigen mixture was added, and after the tubes had been incubated for one to two hours at room temperature and then centrifugated at 1,400 revolutions per minute for three minutes the tests were read. Autoantibodies could be demonstrated during a period of between eight and fourteen days after the onset of the infection. Autoantibodies to rat heart were demonstrated in the serums of 18 of the 46 rats (barring reactions in serum dilutions up to 1:20, the specificity of which is questionable). There was no marked difference in effectiveness between the various mediums and vehicles used for establishment of the focal infec-

8. Footnotes 1, 2 and 7.

tions. However, the incidence of reactions was much lower in serums of the rats given injections of killed (acetone-precipitated) organisms, even when large doses were employed.

Autoantibodies giving definite in vitro reactions with extract of rat kidney were demonstrated in the serums of 6 rats. These serums failed to react with extract of rat heart.

With the use of two pools, each composed of 6 serums reacting with extract of rat heart, about equally strong reactions were obtained with extracts of heart, skeletal muscle and connective tissue, while practically no reaction was observed with extracts of rat lung, liver, brain or kidney.

It must be pointed out that, on the whole, all of these reactions were much weaker than those obtained after rats had been immunized with mixtures of streptococci and rat tissues (heart, connective tissue, etc.), which have been reported elsewhere.[2]

Histologically, significant cardiac or renal lesions occurring as a result of a pathogenic effect of the autoantibodies formed were not observed. However, 2 of the rats showing autoantibodies to kidney did exhibit slight urinary changes consisting chiefly of mild proteinuria and some microscopic hematuria; in 1 of these the changes persisted for a few months but finally subsided.

COMMENT

According to the results reported, it appears that in rats focally infected with group A beta hemolytic streptococci autogenous tissue antigens are formed in consequence of some effect of the streptococci or their toxins and are released into the blood stream. These tissue antigens can be demonstrated by means of potent antiserums to rat tissues produced in the rabbit. Further, as a response to these autogenous antigens, autoantibodies are formed, demonstrable by the use of extracts of rat tissues as test antigens.

In the studies reported it was not possible to decide from what organs or tissues these autoantigens originated. The antikidney serum by means of which the most definite in vitro reactions were obtained reacted strongly not only with rat kidney but also with rat heart. After this serum had been absorbed with rat heart, its reactivity was limited to kidney, but the autogenous antigen in rat serum could not be definitely demonstrated with this absorbed serum. This apparent removal of the antibodies to the antigen in question by heart absorption of the antikidney serum seems to indicate that this antigen is one which is present not only in kidney but also in other tissue such as heart. The fact that the rabbit anti-rat-heart serum failed to yield definite reactions with the autogenous antigen does not necessarily conflict with this view, as this failure may be due to the fact that this serum was markedly weaker than the antikidney serum.

The results obtained with the serum samples containing autoantibodies to the autogenous antigen also support the view that the latter was derived, at least in most instances, from the heart or other tissues containing the same antigen, rather than from the kidney, in that the majority of the reactive serums showed reactions with extracts of heart rather than kidney. It should, however, be mentioned again that some of the serums reacted with kidney and not with heart and that the anti-kidney serum that had been absorbed with rat heart gave slight reactions with a few of the rat serums containing autogenous antigens. In these cases it may be that the latter originated from the kidney.

When the results of the tests for autogenous antigens and autoantibodies were compared with respect to each individual rat, no very definite correlation was evident; i. e., rats whose serum showed relatively strong reactions in tests for autogenous antigen did not always develop autoantibodies in demonstrable amounts and vice versa. But in view of the relatively weak reactivity of the serums on the whole, this inconsistency is not surprising.

Although the evidence presented for the phenomenon described is not very strong, especially in the sense that the intensity of the serologic reactions obtained did not far exceed the minimum detectable by the method employed, it should be pointed out that these experiments were carried out in three different sets at different times and that each time essentially the same results were obtained.

The results of the experiments reported lend further support to the view that autoantibodies to certain tissues may play a role in the genesis of human glomerulonephritis and rheumatic fever. They seem to indicate in principle the existence of the possibility that during the streptococcic infection preceding these diseases autogenous tissue antigens are activated by the effect of streptococcic substances on the tissues of the host. Experimental evidence that, as a response to antigenic tissue-streptococcus combinations, autoantibodies are formed and, owing to the pathogenic effect of the latter, renal and cardiac lesions develop, has already been presented in preceding papers.

The phenomenon described here may have some resemblance to the findings in rheumatic fever, reported by Coburn and Pauli,[9] and further studied by Wedum and Wedum,[10] according to which an antigen was present in the serum of rheumatic subjects during the prerheumatic phase, the antibody to which appeared at the onset of the rheumatic attack. One difference, however, is that in the present experiments the autogenous antigen appeared during the height of the streptococcic infection, while the antigen reported by Coburn was present at a time two to

9. Coburn, A. F., and Pauli, R. H.: J. Exper. Med. **69**:143, 1939.

10. Wedum, A. G., and Wedum, B. G.: Proc. Soc. Exper. Biol. & Med. **61**: 432, 1946.

three weeks after the onset of the streptococcic infection, i. e., in most instances after subsidence of the acute infection and preceding the onset of the rheumatic attack by only a few days.

SUMMARY

In rats focally infected with group A beta hemolytic streptococci, autogenous tissue antigens appear to be released into the blood stream during the height of the infection. These antigens were demonstrated by means of antiserums to rat tissues produced in the rabbit.

As a response to these autogenous antigens, autoantibodies are formed, as demonstrated by the use of extracts of rat tissues as test antigens.

CORRELATIONS OF BIOCHEMICAL AND HISTOLOGIC CHANGES IN THE ADRENAL CORTEX

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THE IMPORTANT functions of the cortex of the adrenal gland have made it the subject of increasingly extensive investigation. As the individual hormones of the cortex have been isolated and their physical and chemical characteristics established, attempts have been made to use histochemical technics to demonstrate their sites of production and storage, and to relate these observations to the activity of the various zones of the cortex. However, the specificity of the histochemical methods used and their correlation with the biochemical changes occurring in this gland have not heretofore been adequately evaluated.

The histologic methods that have been proposed for the detection of biologically active steroids include staining with phenylhydrazine,[1] use of Schiff's reagent,[2] digitonin precipitation and use of reagents of the Leibermann-Burchard test.[3] Estimations have also been made of the amount of birefringence and autofluorescence of the adrenal cortex as examined by polarized light and ultraviolet rays, respectively,[4] and sudan dyes have been used in the estimation of the lipoid content of the adrenal glands for many years.

In 1943 Sayers and associates[5] reported a decrease of adrenal cholesterol following administration of an adrenotropic extract of the pituitary gland. This interesting observation facilitated the interpretation of the previously known fact that the cholesterol content and the

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1. Bennett, H. S.: (*a*) Proc. Soc. Exper. Biol. & Med. **42**:786, 1939; (*b*) Am. J. Anat. **67**:151, 1940.

2. Feulgen, R., and Voit, K.: Arch. f. d. ges. Physiol. **206**:389, 1924.

3. (*a*) Dempsey, E. W., and Bassett, D. L.: Endocrinology **33**:384, 1943. (*b*) Dempsey, E. W., and Wislocki, G. B.: ibid. **35**:409, 1944.

4. Deane, H. W., and Greep, R. O.: Am. J. Anat. **79**:117, 1946.

5. Sayers, G.; Sayers, M. A.; White, A., and Long, C. N. H.: Proc. Soc. Exper. Biol. & Med. **52**:200, 1943.

sudanophilic material of human adrenal glands vary greatly under different circumstances,[6] in general being low in patients with severe infections, moderately decreased in those with chronic debilitating diseases and cancer and high in people who die of cardiorenal disorders.

During the last two years we have determined the cholesterol content of the adrenal glands in selected cases coming to autopsy and have studied these glands with some of the previously mentioned histochemical technics. In this paper, the observations made histologically are compared with the cholesterol contents of the adrenal glands, and an attempt is made to evaluate the significance of the findings.

MATERIAL AND METHODS

The adrenal glands used in this study were from the Mallory Institute of Pathology; some of the glands were supplied by Dr. T. Leary, medical examiner for Suffolk County, Southern District. The adrenal glands were removed intact from the body. The surrounding fat was carefully trimmed off, and the glands were weighed on a torsion balance. Blocks were then cut and placed in a 4 per cent aqueous solution of formaldehyde and in Zenker's solution. The remaining portions of the glands, usually 80 per cent of the total weight, were kept at —10 C. until the determinations of the total cholesterol were done by the Schoenheimer-Sperry method as modified by Sperry.[7] The tissue fixed in Zenker's solution was stained with phloxine and methylene blue. Three frozen sections, each 12.5 to 15 microns thick, were cut from the formaldehyde-fixed blocks and were mounted with glycerin jelly, one being unstained, another stained with sudan IV and hematoxylin and the last stained with phenylhydrazine hydrochloride as described by Bennett.[1b] The unstained frozen section was examined by polarized light and ultraviolet rays.

OBSERVATIONS ON NORMAL ADRENAL CORTEX [8]

The microscopic picture of a normal cortex stained with phloxine and methylene blue or hematoxylin and eosin presents three distinct zones, namely, the zona glomerulosa, the zona fasciculata and the zona reticularis (fig. 1 *A*). The outer one or zona glomerulosa is characterized by a moderately thin layer of cells arranged in well circumscribed clusters. The nuclei of these cells stain fairly dark, while the cytoplasm appears

6. Wacher, L., and Hueck, W.: Arch. f. exper. Path. u. Pharmakol. **71**:373, 1943. Landau, M., and McNee, J. W.: Beitr. z. path. Anat. u. z. allg. Path. **58**: 667, 1914. Chauffard, A.; Laroche, G., and Grigart, A.: Compt. rend. Soc. de biol. **76**:529, 1914. Kohno, T.: Folia endocrinol. japan. **4**:60, 1928. Ewart, B.: Upsala läkaref. förh. **40**:536, 1934-1935. Sarason, E. L.: Arch. Int. Med. **71**:702, 1943.

7. Sperry, W. M.: Am. J. Clin. Path. (Tech. Supp.) **2**:91, 1938.

8. One must be most careful in the histologic interpretation of normal adrenal glands, for those of vigorous young adults dying of diseases seemingly unrelated to the adrenal cortex will show histologic changes consistent with overactivity of the cortical cells. In this series, normal glands have been considered to be those taken from persons who suffered instantaneous traumatic death and showed at autopsy no lesions other than those caused by the accident.

fluffy and vacuolated. In the outermost portion of the zona glomerulosa, just beneath the capsule, there are frequently smaller cells with dark nuclei and acidophilic cytoplasm. The zona glomerulosa blends into the zona fasciculata, which in a normal adrenal gland is the widest zone and is composed of moderately large polyhedral cells arranged in cords. The nuclei of these cells are larger and lighter staining than those of the glomerulosa. The cytoplasm is fluffy and vacuolated, and these cells have been termed "spongiocytes." The vacuolated appearance of both the zona glomerulosa and the zona fasciculata in sections prepared from paraffin blocks results from the dissolution of the lipoid substances in the process of preparation. The line of demarcation between the zona fasciculata and the inner layer or zona reticularis is not distinct. As the reticular zone is approached there is a gradual transition to cells arranged in an irregular fashion, which have acidophilic cytoplasm and various patterns of nuclei ranging from dark staining or pyknotic nuclei to those with a vesicular character. The cells of this layer lack the vacuolated appearance of those of the other zones and frequently contain a brownish yellow pigment, the function of which is not known.[9]

Birefringence.—As seen in figure 1 *B* and *C*, the birefringence of the normal adrenal cortex is marked. Its concentration is almost entirely limited to the zona glomerulosa and the zona fasciculata, particularly the latter. In tissue fixed in formaldehyde solution, the doubly refractile material is in the form of needle-like crystals, although portions may appear diffusely as fine particles. If the slide is heated for a few seconds over a microscope lamp and then gentle pressure is exerted on the cover slip and the slide allowed to cool, the predominant portion of the birefringent material appears as maltese crosses (fig. 2 *A*), which are characteristic of such substances as cholesterol esters.[10]

Phenylhydrazine and Sudan Stains and Autofluorescence.—Figure 2 *B* shows a normal cortex stained with phenylhydrazine hydrochloride. The cells of the zona glomerulosa and the zona fasciculata, particularly those in the outer portion of the latter, appear light yellow. The zona reticularis is slightly yellow, but the medulla remains unstained. In addition, the fat contained in signet ring cells outside the capsule is also yellow. Figure 2 *C* shows the appearance of the same adrenal tissue when stained with sudan IV.

Under ultraviolet rays the adrenal cortex fluoresces yellowish green, the areas of greatest fluorescence being located in the glomerular and fascicular zones. However, because of the diffusion of the light of the fluorescent material, the effort to localize the material in question exactly to the respective areas is more difficult with this method of study.

9. Blackman, S. S.: Bull. Johns Hopkins Hosp. **68**:180, 1946.

10. White, C. P.: J. Path. & Bact. **13**:3, 1909.

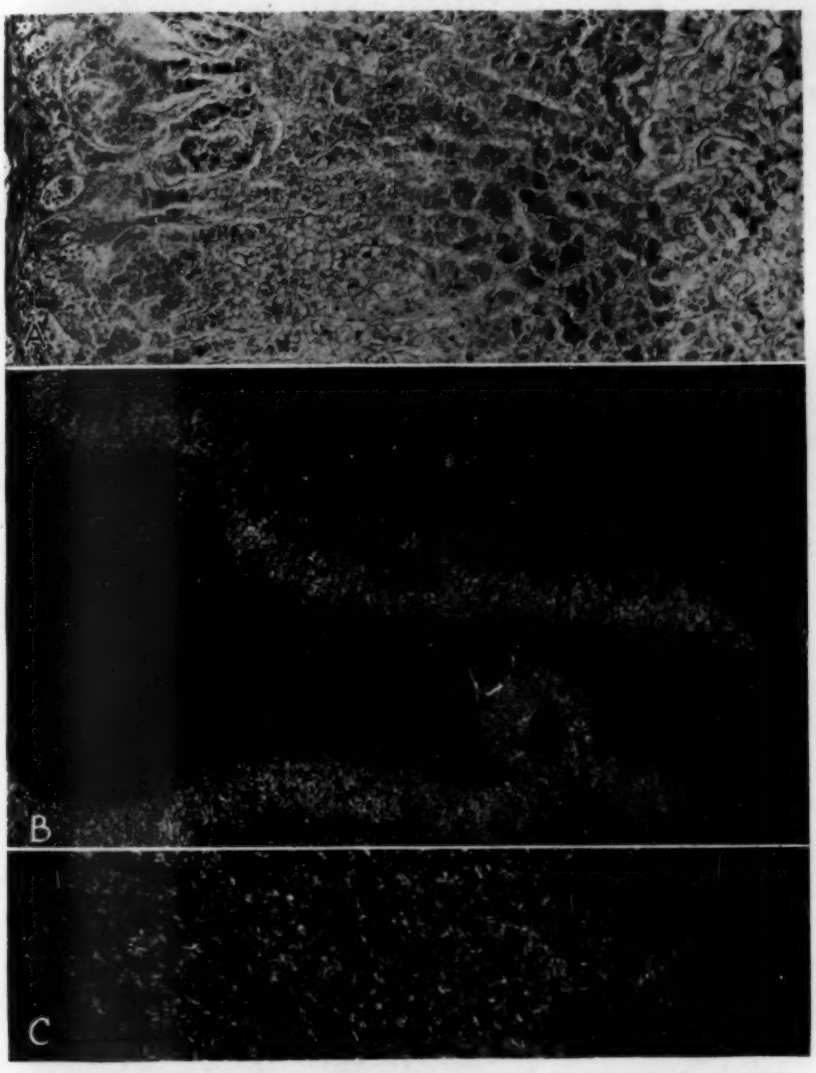

Fig. 1.—*A*, normal adrenal cortex from a 24 year old woman dying instantly of a severed spinal cord. Note the vacuolation and the fluffiness of the cells, particularly of those of the fascicular and glomerular zones. Phloxine–methylene blue; × 127.5. The photomicrographs in figures 1 and 2 are all from sections of the same adrenal gland.

B, normal adrenal cortex examined by polarized light. The birefringence is limited almost entirely to the fascicular and the glomerular zone, particularly to the former.

C, same adrenal cortex as shown in *B* under higher power; × 127.5. The capsule is on the left, while the reticular zone and the medulla are on the right.

If one compares figures 1 *A* and *C* and 2 *B* and *C*, the parallelism of the findings by the various methods is readily appreciated.

OBSERVATIONS ON ADRENAL CORTEX DEPLETED OF LIPOID CONTENT

Whenever the body is subjected to a noxa, whether infection, chemical or physical trauma, roentgen radiation, forced muscular exercise, anoxia or extremes of temperature, the adrenal cortex undergoes characteristic changes.[11] These consist of a depletion of the lipoid con-

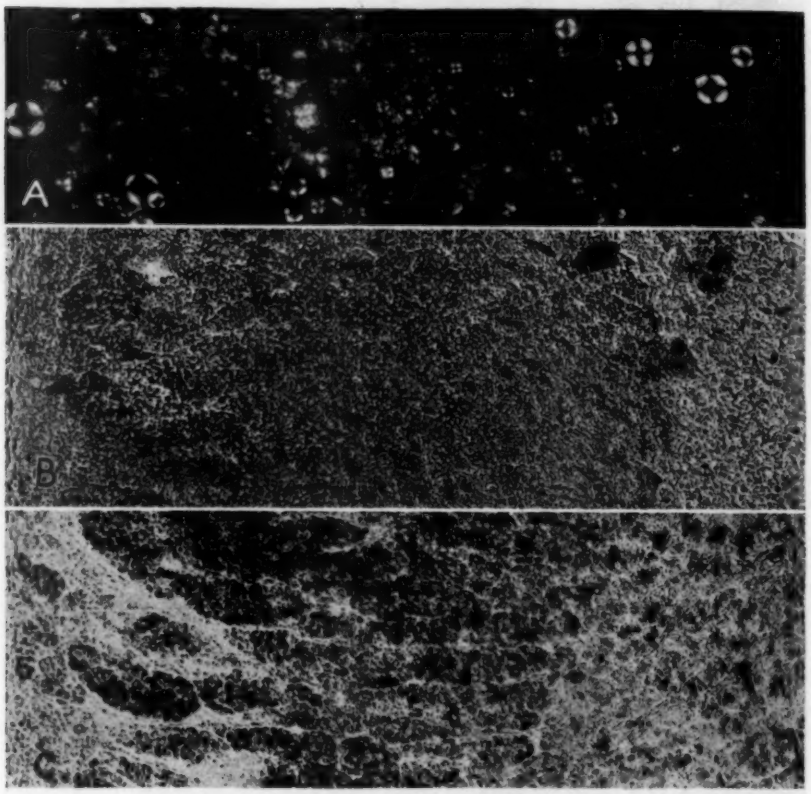

Fig. 2.—*A*, birefringent material of a normal adrenal cortex after application of heat and pressure. Maltese cross formation is characteristic of cholesterol esters. × 127.5.

B, normal adrenal cortex stained with phenylhydrazine. × 127.5. This photomicrograph was made with a blue filter. The darker shade is imparted by the yellow color of the phenylhydrazine. Note that its greatest concentration is in the glomerular zone and the outer part of the fascicular zone. The reticular zone is a lighter yellow, and the medulla remains unstained.

C, normal adrenal cortex stained with sudan IV; × 127.5. Note that the distribution of the stain is similar to that of the birefringence and the phenylhydrazine reaction shown in figures 1 *C* and 2 *B*.

11. Selye, H.: J. Clin. Endocrinol. **6**:117, 1946.

tent, an enlargement and an increase in weight. These phenomena have been interpreted as evidence of increased activity of the cortex, with production of large amounts of corticoid substances, and studies have shown that during periods of "alarm" increased amounts of corticoid substances are excreted in the urine.[12]

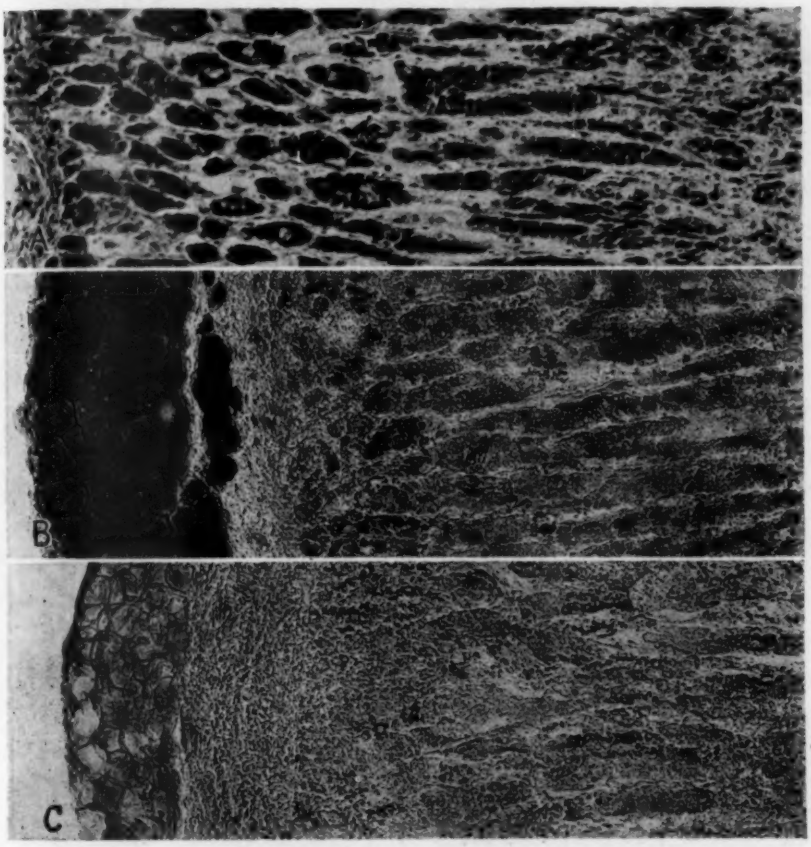

Fig. 3.—*A*, adrenal cortex from a 63 year old man who died of a fulminating streptococcic infection of four days' duration. Phloxine-methylene blue; × 122. Compare with figure 1 *A* and note the absence of vacuolation and the dark staining of the cytoplasm characteristic of an adrenal gland which has been depleted of lipoid material. The photomicrographs shown in *B* and *C* are from this same adrenal cortex.

B, "Lipid-depleted" adrenal cortex stained with sudan IV; × 122. For comparison note the concentration of stainable lipid in fat cells outside the capsule.

C, "Lipid-depleted" adrenal cortex stained with phenylhydrazine; × 122. Note absence of reaction in the cortex and its presence in fat cells. This photomicrograph was made with a blue filter.

12. Weil, P. G., and Browne, J. S. L.: Science **90**:445, 1939. Venning, E. H.; Hoffman, M. M., and Browne, J. S. L.: Endocrinology **35**:49, 1944. Shipley, R. A.; Dorfman, R. I.; Buchwald, E., and Ross, E.: J. Clin. Investigation **25**:673, 1946.

The characteristic picture of the cortex after it has been placed under extreme stress is shown in figure 3. When stained with phloxine and methylene blue, the cells of the zona glomerulosa and the zona fas-

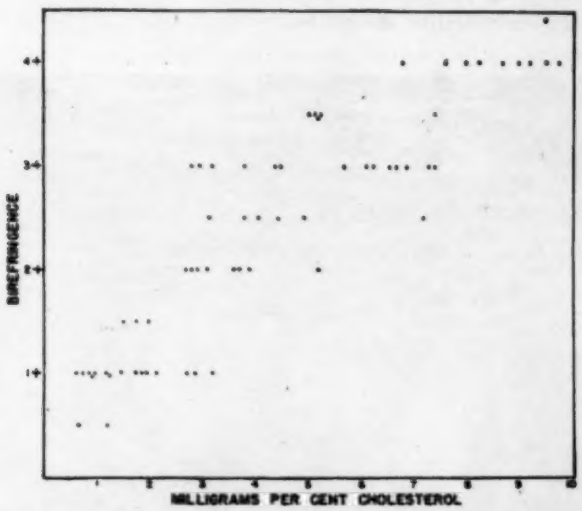

Fig. 4.—The degrees of birefringence plotted against the cholesterol contents of 64 adrenal glands. Note the relationship in this and the two following charts.

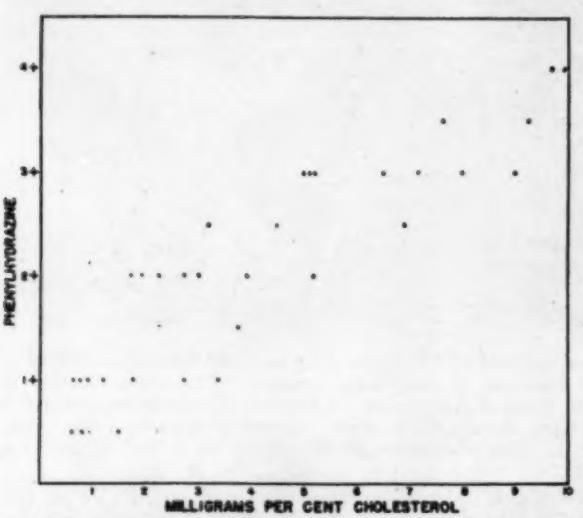

Fig. 5.—The degrees of intensity of phenylhydrazine reaction plotted against the cholesterol contents of 34 adrenal glands.

ciculata are not vacuolated, having lost their lipoid content, and are stained deeply with phloxine (fig. 3 *A*). The cells appear more compact, while the cords are separated by wider sinusoids and spaces. At times

there is distinct tubular formation among the cells. These changes have frequently been described as resulting from postmortem deterioration, but Dr. N. Zamcheck, of the Army Institute of Pathology, observed in his examination of more than 5,000 adrenal glands that in cases of sudden accidental death the length of time post mortem did not alter significantly the normal histologic appearance. In addition, normal adrenal glands may be left at room temperature for as long as fifty-six hours without development of the changes seen in adrenal glands that have been "depleted" of lipoid substance following great physiologic stress.[13]

When an adrenal gland thus depleted is examined by polarized light, there is no visible birefringent material, and under ultraviolet rays the

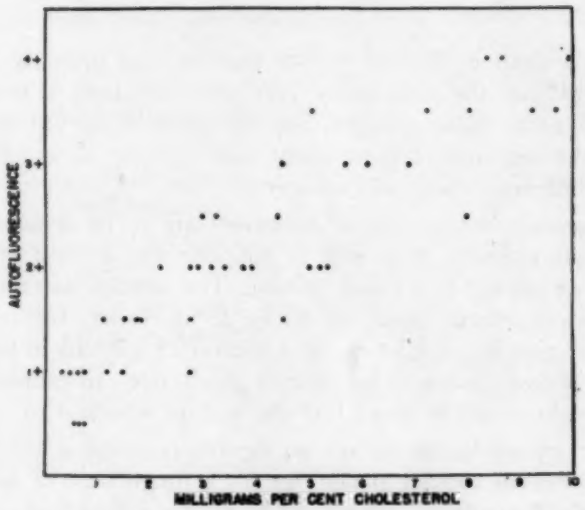

Fig. 6.—The degrees of autofluorescence plotted against the cholesterol contents of 42 adrenal glands.

cortex has a negligible amount of fluorescence. After staining with sudan IV the cortex is completely devoid of sudanophilic material (fig. 3 *B*), and likewise there is no staining with phenylhydrazine hydrochloride (fig. 3 *C*). There are, of course, varying degrees of depletion between the normal cortex and one that has become extensively depleted, but all methods show to an equal degree not only the amount but also the location of the changes.

These results suggest that the methods used merely demonstrate fluctuations in the lipoid content of the adrenal cortex. If this is true, it should be possible to correlate the concentration of total cholesterol

13. Zamcheck, N.: Personal communication to the authors.

with the intensity of the various reactions, as it was shown by Popjak [14] that cholesterol and its esters account for most of the change in the lipoid content of the adrenal gland. Such correlations are shown in figures 4, 5 and 6, in which the amount of birefringence, the intensity of staining with phenylhydrazine hydrochloride and the amount of autofluorescence, respectively, are graded from 0 to 4 plus and plotted against the total cholesterol content of the adrenal glands.

In all three figures the correlations are apparent. As the cholesterol content of the gland decreased, so did the intensity of the other reactions. It was also observed, and should be emphasized, that staining with sudan IV is a reliable index to the concentration of cholesterol and the location and the amount of lipoid material.

COMMENT

In the evaluation of these various methods, the principal aspects to be considered are the presumably very small amounts of cortical hormones present in tissue sections, the nonspecificity of the stains used, including the reactions of these stains with cholesterol, and the significance of a decrease of adrenal cholesterol.

If biologically active adrenal hormones are to be demonstrated by histochemical technics, it is well to consider the amount of hormone postulated as present in a tissue section. The average measurements of a normal adult adrenal gland are 50 by 25 by 8 mm., with each gland weighing an average of 4.5 Gm. If a section 15 microns in thickness is cut in the widest portion of an adrenal gland, one can estimate that its average weight would be about 1.35 mg. and its volume 3 cu. mm.

To our knowledge there are no figures concerning the hormonal contents of human adrenal glands; so, for a rough scale of comparison, the values for beef adrenal glands will be used.[15] Olson and associates [16] did extensive studies on the assay of extracts of adrenal cortex and in four potent extracts of beef adrenal glands the average glycogenic potency was equivalent to 8 micrograms of corticosterone per gram of fresh tissue and the renal function potency was equivalent to 4.5 micrograms of desoxycorticosterone acetate per gram of fresh tissue. At 12.5 micrograms per gram of fresh tissue, the amount of hormonally active steroid one would expect to find in an average section of human adrenal gland 15 microns in thickness would be 0.017 microgram. This amount of material would be spread over an area of approximately 200 sq. mm.

14. Popjak, G.: J. Path. & Bact. **56**:485, 1944.

15. When this comparison is made, it is realized that there are distinct species differences in the concentration of cortical hormones per gram of fresh adrenal tissue (e. g., pig as compared with beef).

16. Olson, R. E.; Jacobs, F. A.; Richert, D.; Thayer, S. A.; Hopp, L. J., and Wade, N. J.: Endocrinology **35**:430, 1944.

In comparison, the cholesterol content of a normal human adrenal gland [17] is about 7 mg. per hundred milligrams of tissue, which results in a postulated cholesterol content of 94.5 micrograms per tissue section, or over five thousand times as much cholesterol as estimated active cortical hormone. The probable difficulty of seeing cortical hormones is emphasized by the fact that when the cholesterol content of human adrenal glands drops below 0.5 mg. per hundred milligrams of tissue, it is difficult to see any birefringent material. At this level there is still 6.8 micrograms of cholesterol per tissue section. Accordingly, if one cannot see this concentration of a known amount of birefringent material, it is difficult to conceive how one could detect 0.017 microgram of birefringent material, the postulated concentration of cortical hormone.

Admittedly, chemical extraction is probably inefficient at present, but if cholesterol at a concentration of four hundred times that of cortical hormone cannot be seen, and, by this token, the chemist is extracting only 0.25 of 1 per cent of the active material, the foregoing observations concerning the improbability of seeing cortical hormones would still be valid.

Although the birefringence directly correlates with the cholesterol content of the gland as shown, it is conceivably not due to this constituent alone, particularly in the normal gland. Some of the double refraction may be imparted by phospholipids and other compounds. However, the contribution of such substances to the total birefringence is apparently considerably less than that of cholesterol, as complete extinction of birefringence is noted at low levels of cholesterol despite the fact that the adrenal gland still contains as much phospholipid as it did in the undepleted state.[14] This fact leads one to the conclusion that materials which are birefringent in crystalline form may not be so when in a medium such as a tissue section.

The phenylhydrazine reaction was proposed by Bennett [1b] and has been used by him and other investigators [18] to show the sites of origin and secretion of steroid hormones. However, Gomori [19] pointed out that the phenylhydrazine reaction and Feulgen's leukofuchsin reaction (Schiff's reagent) as used in the so-called "plasmal" reaction [2] had similar sites of localization. Albert and Leblond [20] recently confirmed this. Both groups of investigators showed that the two reactions occurred with many different tissues, particularly with those rich in lipoid material and in such structures as myelin sheaths, tubercles, infarcts, tumors and fat cells themselves. It is the opinion of these

17. Rogers, W. F., Jr., and Williams, R. H.: Unpublished data.

18. Dempsey and Bassett.[3a] Wislocki, G. B., and Bennett, H. S.: Am. J. Anat. **73**:335, 1943.

19. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **51**:133, 1942.

20. Albert, S., and Leblond, C. P.: Endocrinology **39**:386, 1946.

authors that loosely bound aldehydes of high fatty acids are responsible for the reactions. In addition, we have noted in fresh atherosclerotic plaques intense reactions with phenylhydrazine in the same areas which are stained with sudan IV. We have also found that when the cholesterol content of human adrenal glands decreases, predominantly owing to a fall in the cholesterol ester, the intensity of the phenylhydrazine reaction likewise decreases. It thus seems that phenylhydrazine and Schiff's reagent are not reliable for locating ketosteroids, as they both react with certain widespread lipid fractions.

Autofluorescence, reaction with digitonin, and the Liebermann-Burchard test [21] have been suggested as methods of localizing biologically active steroid hormones. However, cholesterol takes part in all of these reactions whether they occur in sections of tissue or in test tubes. For example, cholesterol when viewed by ultraviolet rays fluoresces a light greenish color; its precipitation with digitonin [22] and its reaction with the Liebermann-Burchard reagent [23] in tissue sections are known, and both of these reactions are used in the quantitative determination of cholesterol.[7] Therefore, it seems that these reactions demonstrate chiefly the presence of cholesterol in high concentration rather than active steroid hormones, which are presumably scanty.

When an adrenotropic pituitary extract is administered to rats and guinea pigs, the cholesterol content of their adrenal glands decreases, and, concomitantly, with this change, there is an increase in glycogen in the liver,[24] which is indirect evidence that, accompanying the fall of adrenal cholesterol, there is secretion of adrenal hormones of the corticosterone type. Thus, the cholesterol content of the adrenal gland seems to be of great aid in indicating the physiologic activity of the gland, but until the biochemical mechanism of the production of biologically active steroid hormones and their relationship to cholesterol are established (i. e., until there is a demonstration of cholesterol being transformed into active steroid hormones, or a refutation of the claim that this occurs) the interpretation of the concentration and the location of lipids, particularly cholesterol, must remain limited. This fact, however, does not detract from the value of the investigations which have shown a shift of lipid patterns following such procedures as hypophysectomy and administration of an adrenotropic pituitary extract, biologically active cortical substances and other agents influencing adrenal activity.

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21. Dempsey and Bassett.[3a] Dempsey and Wislocki.[3b]

22. Windaus, A.: Ber. d. deutsch. chem. Gesellsch. **42**:238, 1909; Ztschr. f. Physiol. **65**:110, 1910.

23. Schultz, A.: Zentralbl. f. allg. Path. u. path. Anat. **35**:314, 1924-1925.

24. Sayers, G.; Sayers, M. A.; Fry, E. G.; White, A., and Long, C. N. H.: Yale J. Biol. & Med. **16**:361, 1944. Sayers, G.; Sayers, M. A.; Liang, T. Y., and Long, C. N. H.: Endocrinology **38**:1, 1946.

SUMMARY

A study has been made of the cortices of normal and "lipid-depleted" adrenal glands of man, in which various histologic technics were used, such as staining with phloxine–methylene blue, phenylhydrazine and sudan IV and examination by polarized light and ultraviolet rays.

The changes in the adrenal glands observed with the respective technics were compared with each other and with the quantitative determinations of the cholesterol contents of these glands.

It was found that with all of the technics except the phloxine–methylene blue the changes paralleled one another as to degree and location and that the intensity of reaction was proportional to the cholesterol content.

We have stressed the fact that the histologic methods which have been suggested for the detection of biologically active steroid hormones yield reactions similar to those of cholesterol and that the latter is in high concentrations, while the hormones are presumably in minute amounts.

The methods described can be used to demonstrate the localization and the shift of lipid patterns in the adrenal gland, which in turn give an indication of the amount of activity due to pituitary adrenotropic hormone. However, there is no proof that they indicate the amount or the distribution of active adrenal hormones.

PATHOLOGIC ASPECTS OF LEIOMYOSARCOMA OF THE STOMACH

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AND
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NEARLY all of the reports concerning leiomyosarcoma of the stomach have dealt with the clinical aspects and the differential diagnosis of sarcoma and carcinoma of the stomach. In this paper the pathologic aspects of leiomyosarcoma of the stomach will be emphasized, and a case will be reported.

REVIEW OF THE LITERATURE

To May 1935 the literature contained reports of 371 cases of sarcoma of the stomach. Three authors, Lubarsch,[1] D'Aunoy[2] and Douglas,[3] reported that 30 to 50 per cent of these cases were cases of either leiomyosarcoma or myosarcoma. Most of the remainder belong in the lymphosarcoma group. In an excellent review of the subject sarcoma of the stomach, Pack and McNeer[4] reported that the tumor occurs on either the greater or the lesser curvature, rather close to the pylorus. In few cases has it been found in the fundus of the stomach. Many authors have emphasized the insidious onset. A large portion of the stomach may be involved without serious epigastric distress. Bleeding is a late symptom. The tumor avoids the orifices, so that obstruction is a late manifestation. Typically the tumor grows by direct extension rather than by blood-borne metastases.

D'Aunoy[2] in his review of the subject noted that the tumor may be either endogastric or exogastric and reported that the latter type is much more frequent. With respect to the histologic classifications of cases of sarcoma in the literature, he listed eighteen separate varieties

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From the Department of Pathology of the University of Utah School of Medicine and the Salt Lake County General Hospital.

1. Lubarsch, O., and Henke, F.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1946, vol. 4; cited by Pack and McNeer.[4]
2. D'Aunoy, R., and Zoeller, A.: Am. J. Surg. **9**:444, 1930.
3. Douglas, J.: Ann. Surg. **71**:628, 1920.
4. Pack, G. T., and McNeer, G.: Ann. Surg. **101**:1206, 1935.

of sarcoma of the stomach. The most common are: lymphosarcoma (39 per cent), round cell sarcoma (24 per cent), spindle cell sarcoma (7 per cent), fibrosarcoma (7 per cent), myosarcoma (6 per cent), round and spindle cell sarcoma (2 per cent), leiomyosarcoma (2 per cent) and fibromyosarcoma (2 per cent). He stated in a summary of the pathologic aspects of sarcoma of the stomach that despite the clinical characteristics and the bulky tumor revealed by roentgen examination, only biopsy can make the diagnosis positive. All of the authors agree that sarcoma of the stomach is frequently operable, even though the defect shown on the roentgenogram may be large.[5]

REPORT OF A CASE

M. H., a 57 year old white woman, suffered from persistent gastrointestinal discomfort for ten years before seeking medical advice in June 1945. (The clinical records made prior to her admission to the Salt Lake County General Hospital were supplied to us by Drs. R. T. Richards and V. L. Rees.) Two months earlier she had undergone thyroidectomy for a nontoxic nodose goiter. The gastrointestinal symptoms at that time consisted of epigastric pain, which was sometimes knifelike and was not relieved by food. An occasional backache was noted, and there were several bouts of emesis without blood. In October 1944 the patient noted the onset of persistent epigastric pain slightly different from the previous spasmodic episodes. This pain lasted for three weeks, and a month later there was pain in the left side of the chest. Weakness and loss of weight followed, although the diet was unchanged. In June 1945 the patient was hospitalized because of massive hematemesis. She required five transfusions. A diagnosis of carcinoma of the stomach was made, following roentgen examination. Because of the large defect and immobility of the left leaf of the diaphragm, the carcinoma was considered inoperable, and palliative treatment was instituted. The patient was ambulatory, without evidence of hemorrhage, and in fair health until May 1946, when she again became weak and bedridden. For three weeks prior to admission to the Salt Lake County General Hospital the patient had tarry stools and frequent bouts of hematemesis. One week before admission, even liquids were regurgitated, and gradual swelling of the lower extremities was noticeable. A massive loss of blood on August 11, 1946, brought the patient to the hospital a day later.

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5. Zellhoefer, H. W. K.: Proc. Staff Meet., Mayo Clin. **10**:625, 1935. Glenn, F., and Douglas, E.: Arch. Surg. **33**: 467, 1936. Phillips, J. R., and Adam, G. F.: Am. J. Surg. **35**:125, 1937. Middleton, W. S., and Paul, L. W.: Radiology **28**:486, 1937. Jordan, S. M.: S. Clin. North America **18**:683, 1938. Lahey, F. H.: Lahey Clin. Bull. **1**:4, 1938. Leiomyosarcoma of the Stomach, Cabot Case 25082, New England J. Med. **220**:351, 1939. Mass, M., and Kirshbaum, J. D.: Am. J. Roentgenol. **44**:716, 1940. Horsley, G. W., and Berger, R. A.: Ann. Surg. **112**:22, 1940. Ross, D. E.:Am. J. Surg. **49**:503, 1940. White, R. R., and Walters, W.: Proc. Staff Meet., Mayo Clin. **16**:378, 1941. Lemon, R. G., and Broders, A. C.: Surg., Gynec. & Obst. **74**:671, 1942. Lyons, C. G., and Schneider, M.: Am. J. Roentgenol. **49**:393, 1943. Bassler, A.: Am. J. Digest. Dis. **10**:342, 1943. Schroeder, G. F., and Schattenberg, H. J.: Arch. Surg. **47**:8, 1943. Chaffin, L.: West. J. Surg. **46**:513, 1938. Baumgartner, C. J.: ibid. **47**:27, 1939. Holta, O.: Acta radiol. **24**:166, 1943.

She was extremely pale and semimoribund and was in mild shock, with a blood pressure of 85 systolic and 50 diastolic, a pulse rate of 96 and a temperature of 97.2 F. No lymphadenopathy was discovered. There was a smooth, firm, painless epigastric mass extending down to the level of the umbilicus, and on rectal examination a nodular mass was palpable in the left posterior quadrant of the abdomen. The clinical impression was that of carcinoma of the stomach, carcinomatosis and anemia. The patient rapidly became weaker, passed large amounts of tarry stools and died three days after admission despite supportive therapy.

Autopsy.—Gross Findings: At autopsy the significant features were: an elliptic healed scar over the anterior aspect of the neck, a decrease in the amount of subcutaneous fat and a palpable mass in the epigastrium. In addition there were: moderate pulmonary congestion; tumor nodules 3 to 10 mm. in diameter, in the liver; hydrops of the gallbladder; a large gastric tumor, which compressed the esophagus and was adherent to the spleen, the diaphragm and the left adrenal gland; a subserous leiomyoma of the uterus, and a serous cyst of the left ovary.

The weight of the stomach with the tumor and the distal 5 cm. of the esophagus was 1,080 Gm. The tumor measured 13 by 18 by 4 cm. and occupied the distal half of the stomach, surrounding the pylorus. It extended upward along the lesser curvature to the esophagus and appeared to infiltrate the submucosa of the esophagus, compressing the lumen. Smooth serosa-covered elevations, measuring 2.5 by 3.5 cm., were scattered over the lesser curvature and the posterior surface of the stomach. The tumor projected irregularly into the lumen, and the mucosa over the larger nodules was ulcerated. The stomach proximal to the tumor was dilated so that the fundus was 15 cm. in length and 17 cm. in circumference. Section of the tumor revealed a gray-white surface with irregular nodules of various sizes. Small soft yellow areas appeared to be necrotic, and here and there were small hemorrhages. It was assumed that the lesser curvature of the stomach was the site of origin, since it was entirely infiltrated by the tumor, while the greater curvature of the stomach was involved only at the most distal portion of the stomach. The pylorus was so narrowed that it would hardly admit a 2 mm. probe. At a point immediately proximal to the pylorus the tumor formed a huge mass that filled the lumen. No areas of hemorrhage were seen in the mucosa, and no open vessel was found which would account for the massive hemorrhage.

Microscopic Observations.—The tumor appeared to originate in the muscularis mucosae, with which it blended intimately (fig. 1). The overlying gastric mucosa was atrophic but retained its continuity. Pseudorosettes were seen, and in some places the cells were palisaded about the small blood vessels. Occasionally the nuclei were irregular and large. The characteristics of leiomyosarcoma were shown in the whorling of long spindle-shaped nuclei (figs. 2 and 3). These cells had a moderate amount of pink-staining or red-staining cytoplasm. Some parts of the tumor showed myxomatous degeneration and large spaces filled with red blood cells.

Figure 4 shows a metastatic nodule in the liver. Here the tumor infiltrated the sinuses, causing atrophy of the liver cord cells without marked necrosis. The metastasis in this figure had the same pattern as the primary tumor.

COMMENT

Grossly, this tumor corresponds to the less frequent type of leiomyosarcoma of the stomach described by D'Aunoy as endogastric. This

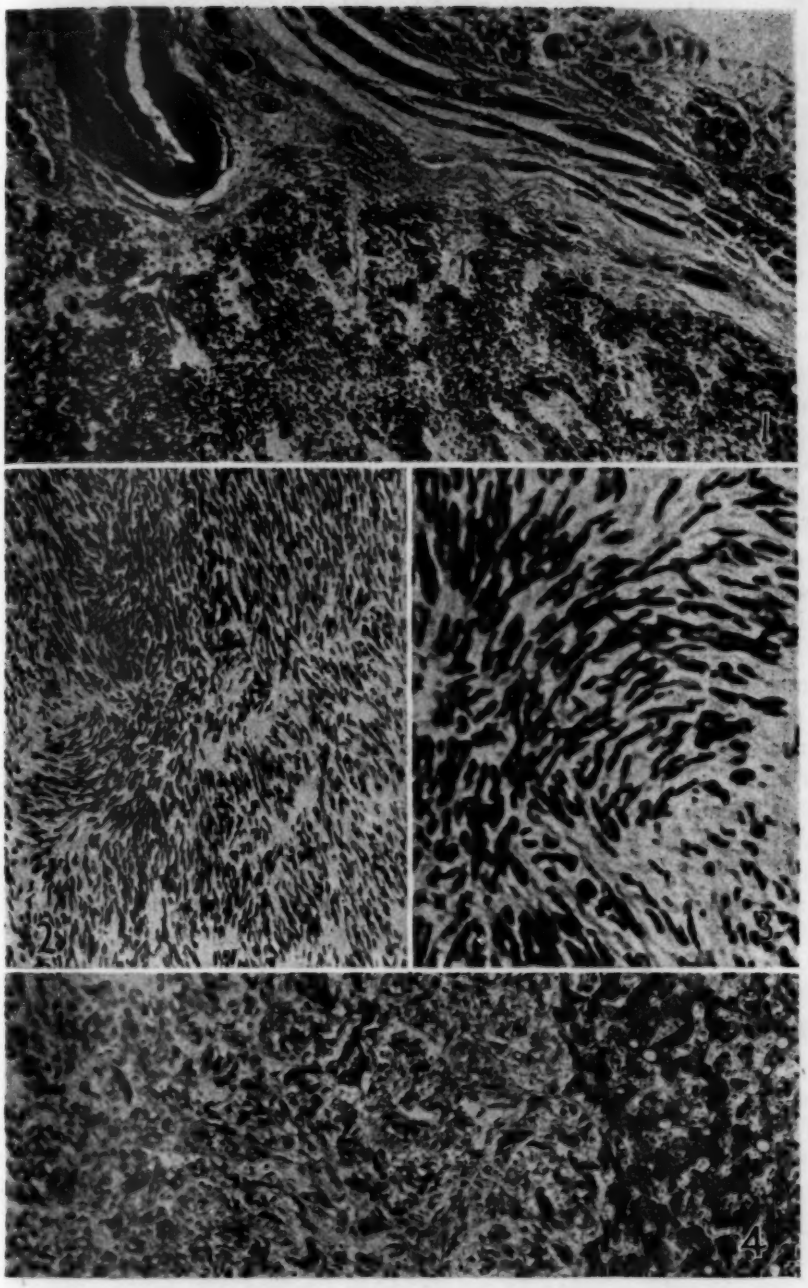

Fig. 1.—Photomicrograph showing leiomyosarcoma occupying the muscularis of the gastric wall. The mucosa is atrophic but not infiltrated by the tumor, which blends intimately with the smooth muscle (approximately × 200).

Figs. 2 and 3.—Low and high power photomicrographs of another histologic pattern of the leiomyosarcoma. The whorling effect is common in spindle cell tumors.

Fig. 4.—Hepatic metastasis. The histologic pattern is maintained (approximately × 200).

type has a tendency to infiltrate the wall beneath the mucosa rather than to penetrate the mucosa and produce an ulcer. After the tumor has reached a certain bulk it projects into the lumen. This particular tumor caused partial pyloric obstruction, although this is unusual for sarcoma of the stomach. The leiomyosarcoma is characteristically formed on the lesser or the greater curvature of the stomach and rarely involves the cardia. This tumor produced the typical clinical syndrome of hematemesis and shock. Here again the hematemesis is seen to be undoubtedly a late clinical manifestation. It is presumed that this tumor had been growing for a long time. Symptoms of the disease appeared two years before death, and it is assumed that the tumor grew for some time before producing any symptoms.

As to the histologic structure of the tumor, we wish to emphasize that several cytostructural variations may be seen in leiomyosarcoma. In one area whorling of the nuclei may be prominent; in another degenerative changes may predominate, with necrosis, hemorrhage and myxomatous changes; in other regions pseudorosette formation may be accentuated, or there may be marked variations in the size and shape of nuclei with giant cell formation and many mitotic figures. Therefore, we feel that the bizarre sarcomatous appearances in the several sections from this tumor represent only variations of one type of tumor, that is, leiomyosarcoma. The various histologic descriptions given in reports of cases in the literature add to the confusion of the microscopic picture, and it might be well to call all of these cases instances of leiomyosarcoma from the demonstrated origin of the tumor in smooth muscle and to speak of other variants as degeneration or predominant cell types. Neurosarcoma and lymphosarcoma have not been mentioned. It is believed that each of these tumors is of a separate origin, with a distinct pathologic pattern of its own.

SUMMARY

A case of leiomyosarcoma of the stomach, with hepatic metastases is reported. The chief gross characteristic of the tumor was the infiltration of the muscularis mucosae with minimum ulceration of the mucosa. The histologic structure included spindle-shaped nuclei, whorling of cells, pseudorosette formation, irregularity of the size of the nuclei and degenerative changes. The confusion of the histologic nomenclature is discouraged, and the term "leiomyosarcoma of the stomach" is preferred for tumors of this type.

RELATIONSHIP OF GROWTH AND NUTRITION TO CARDIORENAL CHANGES INDUCED IN BIRDS BY A HIGH SALT INTAKE

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SODIUM CHLORIDE administered to young, rapidly growing chicks in excess of the permissible 2 per cent of the food intake produces hydrops and cardiac and renal hypertrophy.[1] There is marked enlargement of the renal glomeruli, which is due not only to symmetric growth of the core and the capillaries but to new formation of tufts or papillae.[1c] These arise as papillary outgrowths from the nucleated core, carrying the overlying capillaries with them. Abundant reticulum is deposited within the periphery of such enlarged glomeruli and particularly within the newly formed papillae. The visceral epithelium over the glomerulus proliferates and hypertrophies, with accompanying hyaline and fatty degenerative changes. Renal interlobular arteries and arterioles become tortuous and thick walled but are free from any form of sclerosis.

It was noted in earlier experiments that these morphologic changes appeared to vary with the nutritional status of the chick and with its age at the time when it was first treated with salt. There were two important factors which had to be considered with regard to these variations: (1) the rate of growth of the animal; (2) the effect of an increasing load of salt and water such as is imposed on the rapidly growing bird as a result of the progressive increase of the daily food intake with a fixed concentration of sodium chloride. The following experiments were therefore designed to correlate growth and nutrition with salt and water load in an attempt to determine to what extent each of these factors, or all combined, influence the described cardiorenal changes.

PROCEDURE

One day old New Hampshire chicks were purchased and maintained on a chick starting mash in electrically heated brooders for the first three weeks.

From the Department of Pathology, University of Illinois College of Medicine.

1. (*a*) Selye, H., and Stone, H.: Proc. Soc. Exper. Biol. & Med. **52**:190, 1943; (*b*) J. Am. Vet. M. A. **103**:140, 1943; (*c*) Krakower, C. A., and Goettsch, M.: Arch. Path. **40**:209, 1945.

Thereafter they were placed in individual screened cages and fed a chick growing mash.[2]

In the first five groups of table 1 female chicks were used exclusively. Those in the first four groups were restricted to the experimental diets when they were 28 to 32 days of age. In group 5 the experimental birds had been primed on 3 per cent salt starting mash for the last two weeks in the brooders, before they were given 5 per cent salt growing mash when they were 3 weeks old. A small number of 29 day old cockerels were employed in group 6 and adult cocks 6 months old in group 7.

The composition of the diets fed daily to the different groups of birds is summarized in table 1. Each diet was so constructed as to allow us:

(1) To distribute the salt and water load equally during the day by adding requisite amounts of Cellu Flour.[3] This tended to simulate the eating habits of birds fed ad libitum while permitting us at the same time to maintain a constant concentration of sodium chloride despite the reduced intake of food.

(2) To determine the effect of the daily ingestion of a fixed amount of salt at different caloric levels.

(3) To determine the effect of the daily ingestion of an increasing amount of salt at different caloric levels.

(4) To determine the effect of the concentration of salt in the diet. The concentration of sodium chloride had perforce to be increased in group 2C in which the birds could eat little more than 25 Gm. of the mixture daily and in group 7B, in which adult birds ate a fairly constant amount of food daily. On the other hand, in groups 3B and 6B the concentration of salt was allowed to decrease, although the amount ingested daily was kept constant.

The consumption of food and water was recorded daily. In the determinations of consumed water the amounts lost by evaporation were corrected for. The birds were weighed twice weekly. Food and water were withdrawn from the birds fifteen to eighteen hours prior to the termination of the experiment. The animals were weighed, then anesthetized with chloroform, and blood was withdrawn from the heart. With the blood so obtained, hemoglobin determinations ("Cenco-Sheard-Sanford photelometer"), hematocrit readings (Wintrobe tubes) and chemical analyses were made. The birds were killed immediately after the withdrawal of blood and dissected. In removing the heart, the aorta was regularly sectioned at the level of the junction of the ascending and the transverse portions of the arch. If the epicardium was edematous, multiple incisions were made through it to allow the fluid to escape. The chambers of the heart were opened and emptied of blood. Before the organ was weighed, the epicardial and endocardial surfaces were carefully blotted with filter paper. Before the kidneys were weighed, their external surfaces were likewise blotted dry. To obtain dry weights, the heart

2. These mashes were obtained from the Ralston Purina Mills, St. Louis. The composition of the growing mash ("purina chick growena") is given as protein 17 per cent, fat 3 per cent, nitrogen-free extract 48 per cent and crude fiber 7 per cent, yielding an estimated 388 calories per hundred grams of food. It contains 0.5 per cent iodized salt.

3. Cellu Flour is basically cellulose and has no caloric value. It is supplied by the Chicago Dietetic Supply House, Inc., Chicago.

TABLE 1.—*The Composition of the Daily Diet*

Group	Growing Mash, Gm.	"Cellu Flour," Gm.*	NaCl, Gm.†	Duration Prior to Change, Days	Caloric Value
1 A	6.0	18.0	...	6	23.2
	5.9	17.6	...	..	22.8
B	6.0	18.0	1.0 (4%)	6	23.2
	5.9	17.6	1.5 (6%)	..	22.8
2 A	12.0	11.5	...	..	46.5
B	12.0	11.5	1.5 (6%)	..	46.5
C	12.0	11.5	1.5 (6%)	6	46.5
		↓ decrements of 0.3	↓ increments of 0.3	↓ consecutive 4 day periods	
	12.0	10.0	3.0 (12%)	..	46.5
3 A	18.0	5.5	...	6	69.8
	18.0	12.0	...	4	69.8
		↓ increments of 5.0		↓ consecutive 4 day periods	
	18.0	32.0	...	..	69.8
B	18.0	5.5	1.5 (6%)	6	69.8
	18.0	10.5	1.5 (6%)	4	69.8
		↓ increments of 5.0	↓	↓ consecutive 4 day periods	
	18.0	30.5	1.5 (3%)	..	69.8
C	18.0	5.5	1.5 (6%)	6	69.8
	18.0	10.2	1.8 (6%)	4	69.8
		↓ increments of 4.7	↓ increments of 0.3	↓ consecutive 4 day periods	
	18.0	29.0	3.0 (6%)	..	69.8
4 A	23.5	....	...	..	91.1
B	23.5	....	1.5 (6%)	..	91.1
C	23.5	....	1.5 (6%)	6	91.1
	23.5	4.7	1.8 (6%)	4	91.1
		↓ increments of 4.7	↓ increments of 0.3	↓ consecutive 4 day periods	
	23.5	23.5	3.0 (6%)	..	91.1
5 A	Ad lib. av. 34.1	.....	.....	.....	Av. 132.3
B	Ad lib. av. 35.8	.....	Av. 1.8 (5%)	.....	Av. 138.9
6 A	Progressive increase from 30 to 95	.....	.....	Over total period of 35 days	116.4-368.6
B	Progressive increase from 28.5 to 96.5	.....	1.5 (5% decreasing to 1.5%)	Over total period of 35 days	110.5-362.7
7 A	Ad lib. av. 145	.....	.....	Over total period of 38 days	562.6
B	Ad lib. av. 137.2-96.2	.....	Av. 2.8 (2%) 15.4 (12%)	Over total period of 38 days	Av. 532.3-331.0

* See footnote 3, page 2.

† Figures in parentheses represent the concentration of salt.

and the kidneys were kept in an oven at 100 C. until their weights were constant. Representative sections of all organs were fixed both in Zenker's fluid and in 10 per cent formaldehyde solution.

RESULTS

Percentage Loss or Gain in Weight: Daily Salt and Water Intake.—The pertinent data on the first five groups of animals are given in table 2. The percentage loss of weight in group 2 approached that of group 1 despite the fact that the caloric intake in the former was double that of the latter, but this is readily explained by the longer survival period of the birds on the higher "caloric diet." In the same way, the weight gain of the birds given food ad libitum (group 5) would have been appreciably higher if the experiment had not been terminated a week or two earlier than in groups 3 and 4. The loss of body weight was on the whole greater and the gain of weight less in the experimental animals as compared with their respective controls. This was particularly true of those animals on an ascending scale of salt intake. The fact that these differences are not too apparent in the first two groups can best be accounted for by the presence of edema fluid in the experimental animals which could not be corrected for by employing the lowest weight recorded prior to demonstrable ascites or anasarca.

As a basis for comparison of the effects of high salt intake at different nutritional levels, it will be noted that there was more than a 30 per cent loss in body weight over a two to three week period in the first group of birds, a loss of about 25 per cent over a four week period in the second group, maintenance of the original body weight or stability of weight in the third group, a slow increase in weight over a five to six week period in the fourth group and a normal rapid rise in the fifth group.

A uniform salt load was obtained in all five groups when it was expressed as an average of the daily salt intake in grams per hundred grams of body weight. In those on a fixed daily intake of salt it varied within the narrow range of 0.46 to 0.54 Gm., whereas in those on an ascending scale of salt it varied from 0.68 to 0.74 Gm., which only slightly exceeded the value of 0.65 Gm. obtained in birds on 5 per cent sodium chloride fed ad libitum. It is of interest that the birds in group 6 (table 1) that were allowed to grow freely and were given 1.5 Gm. of sodium chloride daily reduced this daily load from 0.42 Gm. per hundred grams of body weight at the beginning of the experiment to 0.17 at its end. Such a low level, as will be indicated in the section on histopathology, is ineffective in either producing or maintaining the characteristic changes observed in rapidly growing chicks and was in fact associated with regression of the lesions produced earlier by the higher percentage level. In the case of the fully grown cocks in group 7 B (table 1), on the other hand, the value for the average daily salt intake per hundred grams of body weight rose from 0.21 Gm. when

TABLE 2.—*Loss or Gain in Body Weight and Daily Salt and Water Intake*

Group	Diet	Birds	Age at Onset of Experiment, Days	Duration of Experiment, Days	Initial Weight, Gm.*	Final Weight, Gm.*	Loss or Gain in Weight, %	Av. Daily Salt Intake, Gm.† / Body Weight, Gm. ‡	Av. Daily Water Intake, Cc. / Body Weight, Gm. ‡
1A	6.0 Gm. (control).....	8	28	15-20	238.5±22.8	150.1±7.9	−36.7±3.6	....	42.3
1B	6.0 Gm., fixed salt intake.....	9	28	11-17	259.2±27.5	180.0±24.2	−30.5±4.6	0.49	64.7
2A	12.0 Gm. (control).....	6	32	24-32	288.7±31.9	207.7±21.0	−27.6±6.3	....	37.9
2B	12.0 Gm., fixed salt intake.....	6	32	18-28	292.8±23.2	223.5±30.4	−23.8±1.9	0.54	69.0
2C	12.0 Gm., ascending salt intake..	6	32	15-27	314.5±43.5	227.6±41.4	−27.9±3.6	0.74	84.9
3A	18.0 Gm. (control).....	6	32	25-32	298.5±31.1	282.7±35.8	− 4.2±14.9	....	31.4
3B	18.0 Gm., fixed salt intake.....	6	32	25-32	314.3±23.6	302.6±44.5	− 4.1±8.1	0.46	60.0
3C	18.0 Gm., ascending salt intake..	6	32	20-30	311.1±24.1	283.1±38.0	− 7.5±19.8	0.68	83.8
4A	23.5 Gm. (control).....	6	30	31-40	251.8±27.1	375.0±34.4	+49.7±13.9	....	30.2
4B	23.5 Gm., fixed salt intake.....	7	30	31-40	274.4±43.0	391.7±52.4	+43.5±8.8	0.48	67.1
4C	23.5 Gm., ascending salt intake..	7	30	31-40	273.6±24.1	343.3±36.1	+25.4±5.9	0.72	89.8
5A	Ad lib. (control).....	6	21	24	180.3±16.9	394.8±63.4	+117.8±21.1	....	26.2
5B	Ad lib., 5% salt.....	6	21	24	207.3±20.6	384.0±65.3	+85.2±28.5	0.65	63.7

* When possible and necessary, the weights were corrected for edema by employment of the lowest weight prior to demonstrable ascites or anasarca.
† The salt referred to is that daily added to the food over and above the 0.5 per cent already present.
‡ The ratio is expressed in percentage.

they were on 4 per cent salt to 0.42 Gm. on 12 per cent salt. The latter is more or less equivalent to that ingested by birds with a fixed daily salt intake but restricted in growth. No attempt was made to increase the concentration of sodium chloride in the food beyond 12 per cent in order to match that of the groups on an ascending scale of salt ingestion, inasmuch as food consumption diminished pari passu with increased concentration. This is apparent from table 1 where the food consumption dropped from 137.2 Gm. daily in the early experimental periods to 98.2 Gm. toward the end. It was our intention to maintain these animals at a stable weight and on an adequate intake of food for comparison with group 3 particularly.

Water consumption closely paralleled salt intake. In day by day values and particularly in certain birds in the subgroups on an ascending scale of sodium chloride, the amount of water ingested daily often greatly exceeded the animal's body weight. Although the birds in group 5B, fed an ad libitum diet, consumed as much salt per day as the birds on a reduced caloric intake but with increasing levels of salt, their consumption of water paralleled that of the birds on a fixed daily intake of salt. The reason for this is not clear.

Hemoglobin Values, Hematocrit Readings and Results of Chemical Analysis of Blood.—These values for groups 2 to 5 inclusive are given in table 3. It is of interest that with regard to both range and average the concentration of hemoglobin was essentially the same in all control groups despite starvation. In those given sodium chloride, however, there was a wide range in groups 2 to 4. The very low values for hemoglobin in groups 2 B, 2 C and 3 C are in great part ascribed to profuse intestinal hemorrhage, while the very high values are probably due to dehydration and in some instances to shock. The latter was seen either in association with massive hemorrhage of the intestinal tract or in birds with marked anasarca. Aside from these factors there is little to indicate that high intake of salt per se was instrumental in producing anemia. In fact, despite the marked gelatinous and edematous character of the bone marrow of birds that were rapidly declining in weight, there always appeared to be a little more hemopoietic tissue microscopically in the treated animals than in the controls. It was markedly reduced in both, however. The bone marrow in birds that were maintained at a stable weight in group 3 was still edematous and hypoplastic. In all others it was essentially of normal fatty character, with greater cellularity and activity in treated animals.

The values for nonprotein nitrogen of the blood were practically all within the normal range. The few exceptions were found in the groups whose intake of food was restricted, with the two highest values in those given 12 Gm. of food daily, viz., 60 mg. per hundred cubic centimeters

for a control and 94 mg. for one on an ascending intake of salt. The latter was moribund when it was killed, and there was considerable blood in its intestinal tract. Probably in these later stages of starvation cardiovascular failure, with or without ascites, tended to reduce renal filtration, accounting for the elevated level of nitrogenous products in the blood of some of the birds. It is doubtful whether blood absorbed from the intestinal tract could account for it in most of these cases since, in their debilitated state, the birds died soon after such profuse hemor-

TABLE 3.—*Hemoglobin Values, Hematocrit Readings and Results of Chemical Analyses of Blood*

Group	Diet	Birds	Hemoglobin, Gm. per 100 Cc.	Hematocrit Reading, Vols. %	Blood Nonprotein Nitrogen, Mg. per 100 Cc.	Plasma Protein,† Gm. per 100 Cc.	Plasma Chlorides, Mg. of NaCl per 100 Cc.
2A	12.0 Gm. (control).....	6	8.3-10.8* 9.4	29-36 32.5	12.5-60.0 31.3	0.04-1.7 0.83	635-725 685.8
2B	12.0 Gm., fixed salt intake.....	4	4.5-10.8 7.7	15-34 25	16.5-40.0 28.3	0.17-0.99 0.64	695-840 746
2C	12.0 Gm., ascending salt intake..	3	5.1-8.8 7.1	16-30 23.6	9.3-94.0 39.7	0.50-1.38 0.84	760-900 850
3A	18.0 Gm. (control).....	6	9.1-9.6 9.3	29-34 32	26.4-40.5 32.3	0.82-2.02 1.34	710-750 732
3B	18.0 Gm., fixed salt intake.....	5	6.8-12.7 10.3	22-40 30.6	12.5-26.4 19.9	1.39-2.87 2.00	680-750 706
3C	18.0 Gm., ascending salt intake..	4	2.0-12.3 8.9	8.0-42.5 29.8	9.9-50.0 33.5	0.76-2.72 1.95	600-880 742.5
4A	23.5 Gm. (control).....	5	9.1-10.5 9.9	30-37 32	24.7-32.5 28.1	1.80-3.54 2.56	
4B	23.5 Gm., fixed salt intake.....	5	6.4-9.9 8.6	.....	13.9-30.4 22.0	1.31-2.76 2.23	
4C	23.5 Gm., ascending salt intake..	6	7.6-11.2 9.6	.....	24.7-46.0 31.9	2.50-4.86 3.36	
5A	Ad lib. (control).....	6	7.6-10.8 9.3	30-36 33.8	23.1-39.3 27.4	2.20-3.88 2.88	
5B	Ad lib., 5% salt.....	10	8.3-10.5 9.6	30-37.5 33.8	18.5-33.3 25.3	2.05-4.27 2.96	

* In this and the following columns the top figure represents the range; the lower single figure, the average.
† Method of Campbell and Hanna, modified (J. Biol. Chem. **119**: 15, 1937).

rhage. It is of interest, however, that despite the marked glomerular changes in the treated animals fed ad libitum, there was no evidence of nitrogenous retention.

The plasma proteins graded themselves in accordance with the daily caloric intake. The lowest values in the four groups in which they were determined were obtained in birds given 12 Gm. of food daily. These low values were, however, unassociated with ascites in the controls (table 6), though frequently accompanied by accumulations of such fluid in the treated animals. The same was true to a certain extent for the group fed 18 Gm. of food daily. It is a striking fact that values as low as 0.04 Gm. and 0.27 Gm. per hundred cubic centimeters were unassoci-

ated with edema in the controls, while appreciably higher values were associated with edema in salt-treated animals. It seems also true that in general the plasma proteins were higher in salt-treated than in control animals of all groups, as though a compensatory mechanism had been brought into play whereby plasma proteins were produced to a greater degree or mobilized more freely.

Hyperchloremia occurred chiefly in the subgroups on ascending intake of salt and was more pronounced in the subgroup given 12 Gm. of food daily than in the one given 18 Gm. In some but not all of these instances there was nonprotein nitrogen retention as well as hyperchloremia, strengthening the view already expressed regarding cardiovascular failure and reduced renal filtration.

Blood Pressure and Heart Rates.—The determinations of arterial blood pressure were performed by Mr. W. Glen Moss, of the department of physiology of the University of Illinois. A modified Hamilton manometer was employed. Through a small incision in the unanesthetized bird, the femoral artery was exposed, and direct intra-arterial blood pressure readings were obtained. In view of the relatively small size of the artery, narrow-gaged needles had to be used, which yielded records from which mean pressures could be determined but not systolic or diastolic readings.

It will be seen from table 4 that both young and older birds were employed under a variety of conditions. Young chicks 24 days of age were fed 12, 18 or 23.5 Gm. of food daily, which resulted in loss of body weight, maintenance of body weight or slow gain in weight respectively. Their experimental counterparts were fed sodium chloride on an ascending scale in a manner similar to that outlined in table 1. As far as the young adult birds were concerned, some were fed 20 or 35 Gm. of food daily, with marked loss of body weight at the former level and approximate maintenance of body weight on 35 Gm. The treated animals were retained on 5 per cent sodium chloride.

The findings in 51 birds, comprising a total of 87 observations, are recorded. The average mean pressures were consistently higher in almost all groups of birds on salt than in their respective controls. The greatest differences or highest pressures, however, were obtained in treated young chicks which were allowed to grow slowly, in young adult birds given food ad libitum with 5 and 8 per cent salt and in treated adults on a maintenance diet. It will be noted that there was a lesser decline of blood pressure in treated birds that were sharply restricted in their caloric intake as compared with their controls. Excessive concentration of salt, as in the case of adults on 15 per cent sodium chloride, tended to depress the blood pressure in the same manner as undernutrition.

It is of interest that with regard to heart rates the situation, at least in young birds, was reversed. The rate tended to be slower in treated than in control animals. The differences were, however, slight in adults inasmuch as the heart rate is normally slower in older birds.

TABLE 4.—*Blood Pressure Determinations and Heart Rates*

Diet	Age of Birds, Days	Birds	Observations	Days on Diet	Mean Blood Pressure, Mm. Hg *	Heart Rate per Minute *
12 Gm. food + 11.5 Gm. "cellu flour"	24	4	6	5-14	82-128 112.1	314-564 458
12 Gm. food + ascending 6.0-8.4% NaCl + "cellu flour"	24	3	5	5-12	95-135 114.2	175-435 332
18 Gm. food + ascending "cellu flour"	24	2	4	5-12	95-124 114.2	348-460 3.2
18 Gm. food + 0% ascending NaCl + ascending "cellu flour"	24	3	5	5-12	108-140 121.8	254-456 367
23.5 Gm. food + ascending "cellu flour"	24	2	4	5-12	100-138 114.0	438-464 456
23.5 Gm. food + 0% ascending NaCl + ascending "cellu flour"	24	2	4	5-12	108-145 132.5	344-440 393
Ad lib. (control)	32-38	2	4	....	106-122 113.2	444-486 471
Ad lib. + 3% NaCl	32-38	4	6	On NaCl from 1 week of age	95-130 113.0	376-486 440
Ad lib. + 5% NaCl	32-38	4	5	On NaCl from 1 week of age	100-135 120.0	346-414 378
Ad lib. (control)	90 and up	7	13	....	112-132 126.6	300-440 363
20 Gm. food + 20 Gm. "cellu flour"	90 and up	2	4	34-48	96-105 99.7	300-360 332
35 Gm. food + 25 Gm. "cellu flour"	90 and up	3	5	30-48	75-122 99.0	330-360 342
Ad lib. + 5% NaCl	90 and up	5	9	On NaCl from 1 week of age	116-168 143.1	270-435 367
Ad lib. + 8% NaCl	90 and up	2	2	On NaCl from 1 week of age	150, 150 150	348, 396 372
Ad lib. + 15% NaCl	90 and up	2	4	On NaCl from 1 week of age	105-142 123.7	240-390 315
20 Gm. food + 5% NaCl + 18 Gm. "cellu flour"	90 and up	1	1	On NaCl from 1 week of age; on restricted diet for 34 days	 115	 95
35 Gm. food + 5% NaCl + 22 Gm. "cellu flour"	90 and up	3	6	On NaCl from 1 week of age; on restricted diet for 30-48 days	125-162 139.1	240-330 295

* The top figure represents the range; the single lower figure, the average.

Gross Anatomic Findings.—Despite the profound emaciation of the birds on 6 or 12 Gm. of food daily, there was little anatomic evidence of any specific vitamin deficiency. In some birds fed these restricted diets,

however, plantar hyperkeratoses were present, which ulcerated and bled. This may have indicated a deficiency of biotin or of pantothenic acid. It is also possible that the intestinal hemorrhages so often found in these starved birds might have been due in part to vitamin K deficiency, aside from starvation. Hemorrhages elsewhere, however, were practically never observed. Erosions of the gizzard were rarely seen. Osteoporosis was an almost constant finding in these two groups of birds and occurred occasionally in birds fed 18 Gm. of food daily. Edema of the epicardial tissues with absence of fat was constantly observed in birds fed 6, 12 and 18 Gm. of food, respectively, and not infrequently in those fed 23.5 Gm. of food daily. The degree of epicardial edema was more marked in treated than in control animals.

TABLE 5.—*Gross Autopsy Findings*

Group	Diet	Birds	No. that Died	No. with Intestinal Hemorrhage	No. with Ascites	No. with Hydropericardium
1A	6.0 Gm. (control)	8	8	2	1	2
1B	6.0 Gm., fixed salt intake	9	5	2	4	5
2A	12.0 Gm. (control)	6	..	3	..	1
2B	12.0 Gm., fixed salt intake	6	1	1	4	6
2C	12.0 Gm., ascending salt intake	6	2	3	4	4
3A	18.0 Gm. (control)	6	..	2	..	..
3B	18.0 Gm., fixed salt intake	6	1	1	2	4
3C	18.0 Gm., ascending salt intake	6	1	2	2	4
4A	23.5 Gm. (control)	6	..	..	..	..
4B	23.5 Gm., fixed salt intake	7	..	..	..	3
4C	23.5 Gm., ascending salt intake	7	..	..	1	3
5A	Ad lib. (control)	6	..	..	..	..
5B	Ad lib., 5% salt	6	..	..	..	3
7A	Adult cocks (controls)	3	..	..	..	..
7B	Adult cocks on ascending salt intake	3	..	..	..	1

It is apparent from table 5 that the mortality was particularly high in group 1, diminishing in groups 2 and 3. There was none in the remaining groups. The mortality was greater in treated than in control animals of the first three groups.

Intestinal hemorrhage was observed in the first three groups on 6, 12 and 18 Gm. of food, respectively, and with equal frequency in control and experimental birds. It was either slight or, more often, profuse. The bleeding occurred from multiple punctate areas in the duodenum and upper portions of the small intestine. This seemed to be preceded by erythema of these areas of mucosa as contrasted with the pallor of the mucous membrane elsewhere.

The incidence of ascites was particularly high (66 per cent) in the salt-treated animals given 12 Gm. of food, but occurred in 44 per cent of the birds in group 1B and in 33 per cent of those on either fixed or

ascending levels of salt with an intake of 18 Gm. of food daily. By contrast with experiences in earlier experiments in which hydrops occurred fairly frequently in birds given food ad libitum with 3 per cent sodium chloride, no edema developed in group 5B given a 5 per cent salt diet ad libitum. The better quality of the commercial feed used in these experiments and the season of the year when the experiment was performed have probably had a good deal to do with this. With shorter hours of daylight there appears to be less tendency for anasarca to develop in birds with a high salt intake fed ad libitum than with longer hours of daylight in summer. This may be due to overloading as a result of longer feeding time.

It will be noted, however, that hydropericardium was the commonest gross anatomic finding in all groups of birds on salt.

Heart and Kidney Weights.—The heart weight per unit of body weight was appreciably higher in treated birds as compared with their respective controls in all experiments (table 6). This enlargement was due mainly to increased ventricular capacity with maintenance of, or a variable degree of increase in, myocardial thickness of both right and left ventricles. Statistical analysis reveals that the cardiac hypertrophy was highly significant in all groups except those fed 6 and 12 Gm. of food, respectively. The degree of hypertrophy varied from 31.0 to 62.2 per cent but within these four groups (3, 4, 5 and 7, table 6) was independent of the caloric intake, the salt level or the age of the birds. In the case of the birds given 6 and 12 Gm. of food daily, the heart weights of the treated animals exceeded those of the controls by 16.1 and 18.6 per cent, respectively, as computed on the basis of heart weight–body weight ratios. Nevertheless, from a statistical standpoint the differences in these two groups were not too significant. They do indicate, however, that despite profound loss in body weight, cardiac weight tends to be retained in the animals to which salt has been administered, while it decreases appreciably in the controls. There is no hypertrophy in a strict sense since the average cardiac weights of these experimental animals approximated the weights estimated at the onset of the experiment prior to a loss of 25 per cent or more of body weight.

It was quite different when kidney weights were analyzed statistically. Highly significant differences between experimental and control values were obtained only in birds having 18 Gm. of food and a fixed salt intake and in young pullets and adult cocks fed ad libitum. It is of interest, however, that there was a definite trend in any single group whereby the degree of renal enlargement varied directly with the daily salt intake; i. e., there was a greater degree of renal enlargement in birds on an ascending scale of salt than in those on a fixed daily intake

of salt. In terms of percentage increase in renal weight above their respective controls, those on a fixed salt intake ranged from 11.8 to 34.3, and those on an ascending salt intake, from 27.6 to 55.2. Here, too, the renal enlargement was relative in birds on 6 and 12 Gm. of food daily and on a fixed intake, the weights being approximately equal

TABLE 6.—*Kidney and Heart Weights*

Group	Diet	Birds	Heart Weight / Body Weight*	Dry Heart Wt. / Wet Heart Wt.*	Kidney Weight / Body Weight*	Dry Kidney Wt. / Wet Kidney Wt.*
1A	6.0 Gm. (control)	7	0.62±0.07 0.028	.....	0.92±0.14 0.055	
1B	6.0 Gm., fixed salt intake	4	0.72±0.07 0.035 0.1>P>0.05	.....	1.12±0.14 0.071 0.1>P>0.05	
2A	12.0 Gm. (control)	6	0.59±0.03 0.014	17.6±0.6	0.76±0.07 0.031	21.5±0.7
2B	12.0 Gm., fixed salt intake	5	0.70±0.12 0.054 0.1>P>0.05	16.2±0.2 (3 birds)	0.85±0.16 0.074 0.4>P>0.3	19.6±0.9 (3 birds)
2C	12.0 Gm., ascending salt intake	5	0.70±0.08 0.038 0.05>P>0.02	16.6±1.6 (3 birds)	0.98±0.22 0.100 0.1>P>0.05	19.8±1.4 (3 birds)
3A	18.0 Gm. (control)	6	0.53±0.08 0.034	17.4±0.7	0.68±0.11 0.046	21.7±0.6
3B	18.0 Gm., fixed salt intake	6	0.86±0.15 0.063 P<0.01	17.4±1.3 (5 birds)	0.91±0.10 0.041 P<0.01	21.0±1.2 (5 birds)
3C	18.0 Gm., ascending salt intake	6	0.85±0.15 0.063 P<0.01	16.9±1.4 (4 birds)	1.05±0.37 0.154 0.1>P>0.05	19.4±2.6 (4 birds)
4A	23.5 Gm. (control)	6	0.58±0.05 0.020	21.6±1.9	0.70±0.22 0.091	
4B	23.5 Gm., fixed salt intake	7	0.76±0.11 0.043 P<0.01	21.6±2.9	0.88±0.25 0.095 0.3>P>0.2	
4C	23.5 Gm., ascending salt intake	7	0.85±0.15 0.057 P<0.01	19.0±1.0	1.07±0.22 0.085 0.02>P>0.01	
5A	Ad lib. (control)	6	0.59±0.05 0.022	.....	0.86±0.06 0.027	
5B	Ad lib., 5% salt	6	0.89±0.17 0.070 P<0.01	.....	1.08±0.12 0.047 P<0.01	
7A	Adult cocks (controls)	3	0.45±0.02 0.012	17.7±1.0†	0.41±0.02 0.014	22.9±1.1
7B	Adult cocks on ascending salt intake	3	0.62±0.11 0.064 P<0.01	20.3±0.5†	0.52±0.10 0.060 P<0.01	21.8±0.4

* The ratio is expressed as a percentage. The top figure represents the mean ± the standard deviation of the mean $\left[\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}\right]$.

The single lower figure represents the standard error of the mean $\left[SE\hat{m} = \frac{\sigma}{\sqrt{n}}\right]$.

The *P* values were obtained from Fisher's tables.

† These determinations are on a fat-free basis.

to those estimated for their given body weights at the onset of the experiment, whereas there appeared to be actual hypertrophy in those on an ascending salt intake and 12 Gm. of food.

The differences in dry weights between control and experimental hearts and kidneys were sufficiently insignificant to alter but little the differences in the wet weight of the organs as described.

There were only 4 animals in group 6 (table 1). The average heart weight–body weight percentage of the two controls was 0.46 and that of the two experimental birds 0.60. There was no difference in the kidney weight–body weight percentage of the two groups, which averaged 0.70. Cardiac hypertrophy seemed to be retained in the experi-

TABLE 7.—*Comparison of Renal Microscopic Changes in Experimental Birds and Appropriate Controls**

Group	Diet	Glomerular Size	Glomerular Tufting †	Glomerular Reticulosis ‡	Hypertrophy and Hyperplasia of Visceral Epithelium of Glomeruli	Arterial and Arteriolar Hypertrophy
1	6.0 Gm. food.....	0	0	0	0	1
2	12.0 Gm. food daily.....	0 (1)	0 (1)	1-2	1 (3)	1 (4)
3	18.0 Gm. food daily.....	0-2	0 (1)	1-2	1	2-3
4	23.5 Gm. food daily.....	0-1	0 (1)	1-2	1	1-3
5	Ad lib.....	4	4	2-4	4	4
6	Progressive increase in food intake, with fixed daily salt intake in experimental animals.....	1	1	1	1	1
7	Adult cocks, fed ad lib., with ascending salt intake in experimental animals.....	0-3	0	1-3	4	2-3

* The microscopic changes are graded as follows: 0 signifies no change; 1, slight increase; 2, moderate increase; 3, marked increase; 4, very marked increase. The figures in parentheses represent changes found in an occasional bird but not characteristic of the group.

† Glomerular tufting refers to the new tufts or papillae formed as outgrowths from the core.

‡ Glomerular reticulosis refers to the increased deposition of reticulum noted beneath and between the glomerular capillaries as well as to some extent within the glomerular core itself.

mental animals with little evidence of regression despite the fact that the salt intake per unit of body weight was reduced below 0.2 per cent.

Microscopic Observations.—There was profound visceral and skeletal muscular atrophy in the first two groups of birds on 6 and 12 Gm. of food daily. Muscular dystrophy was encountered in 2 birds, a control on 12 Gm. and a treated animal on 18 Gm. of food and a fixed salt intake. Aside from these there were few findings of note other than those in the kidneys. The changes in arterial vessels detailed in following paragraphs were much less apparent in other organs or tissues of experimental animals. It may be added, however, that in exposing the femoral arteries for determinations of blood pressure we observed that these vessels were wider and fuller in the experimental than in the control animals.

Table 7 presents the changes in glomeruli and renal blood vessels of treated animals, whether these were on fixed or on ascending salt levels, as compared with their respective controls in each of the seven groups outlined in table 1. These changes in the structures referred to pertain to the inner third of the renal cortex and were evaluated qualitatively. It was only in adult birds that the glomeruli of the outer two thirds of the cortex participated in any way in these changes.

Increase in glomerular size was most pronounced in actively growing birds of group 5 (fig. 6). It was slight in those of group 6 which were allowed to grow freely but in which the salt intake per unit of body weight was allowed to decline below 0.2 per cent. In all other groups there was either no change in size, as in those on sharply restricted caloric intakes, or slight to moderate change in the others, with the exception of more marked hypertrophy in one of the adult cocks on an ascending scale of salt (fig. 8).

Glomerular tufting, or papillary outgrowth from the glomerular core, was confined almost entirely to group 5 (fig. 6). There was some persistent tufting of the glomeruli in group 6 in association with the remolding of the glomerulus by growth alone, inasmuch as the stimulus induced by salt was largely ineffective in maintaining or augmenting the hypertrophied state produced in the earlier stages of the experiment when the salt intake per unit of body weight was greater than 0.2 per cent. In all other groups, whatever glomerular enlargement occurred was symmetric in character (figs. 2 and 8), without tufting. There was some abortive tufting in a few birds given restricted diets and ascending salt levels, with or without some degree of glomerular enlargement. This was seen in birds with the highest heart weight–body weight ratios. In such instances the widened glomerular capillaries had at times a rigid, wire loop appearance, associated with some thickening of the basement membrane, but largely the result of pericapillary reticulosis.

Increased amounts of reticulum were deposited beneath and about the glomerular capillaries, as well as to some extent in the core itself, to a variable degree in all treated birds except those given 6 Gm. of food daily. It was most pronounced in groups 5, 7 and the few exceptions with abortive glomerular tufting in the calorically restricted groups. It was least pronounced in group 6.

Hypertrophy and hyperplasia of the visceral epithelium of the glomerulus occurred to about the same degree and under the same circumstances as reticulosis.

The slight arterial and arteriolar changes in groups 1 and 2 represented better preserved, i. e., less atrophic vessels rather than true hypertrophy. The thicker muscular fibers of the medial coat appeared

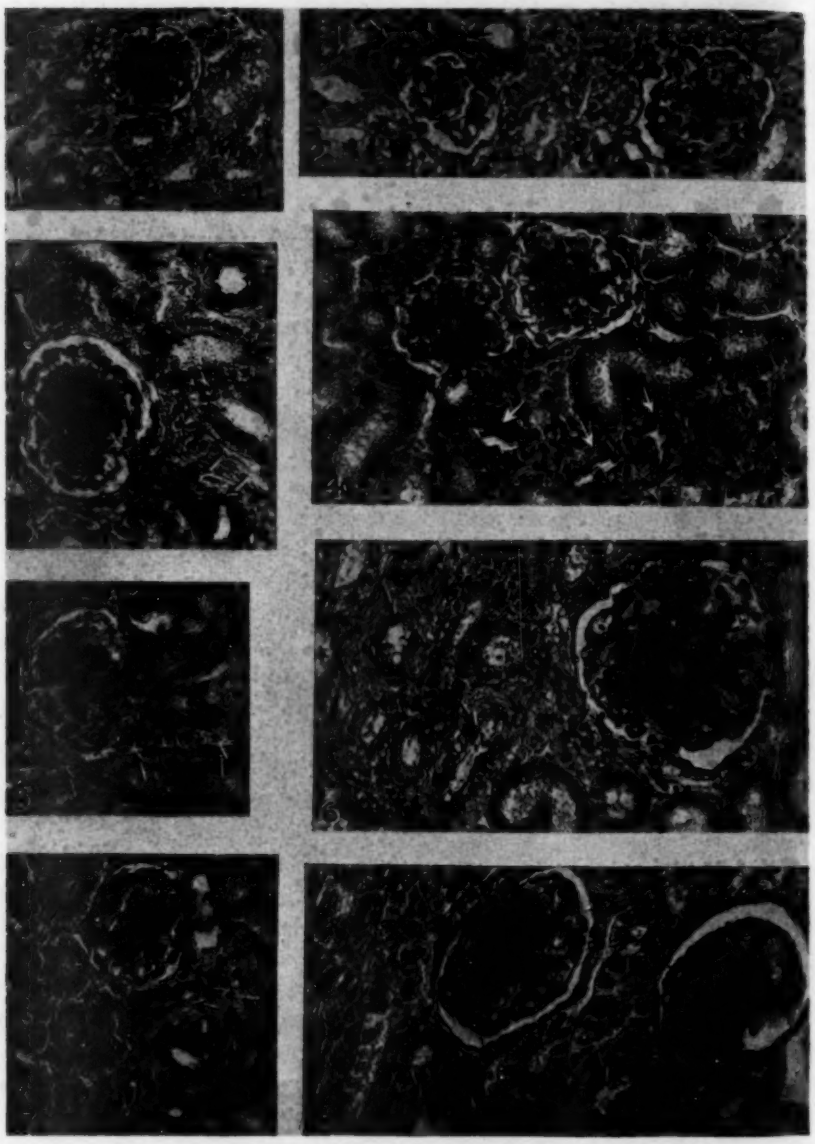
somewhat hydropic, and their larger nuclei were more vesicular. In all other groups except group 6 there were pronounced changes in these vessels. They were markedly tortuous, with thick-walled, frankly hypertrophied medial coats, out of proportion to the increased size of the kidney and not necessarily associated with glomerular hypertrophy (fig. 4). Neither intimal sclerosis, whether fibrosis or hyalinization, nor changes in the elastic lamellas, such as splitting, were observed. The arterial vessels in the experimental animals of group 6 were only slightly thicker and more tortuous than those of the controls, indicating here, again, the adaptation of previously hypertrophied vessels to the enlarging kidney as a result of growth, but that they were progressively more removed from the influence of the effects of sodium chloride.

There were certain other differences which merit comment. In rapidly growing birds with a high salt intake the following abnormalities are commonly found: hyaline droplet and fatty changes in the hypertrophied visceral epithelium of Bowman's capsule and mitotic activity of the hyperplastic epithelium; deposition of lipids in the subcapillary layers and tufts of the glomerulus; droplets of fat in tubular epithelium, particularly in that of regenerated sectors; the presence of red blood cell casts and associated with them regenerated sectors of tubules. Such changes were almost entirely lacking in treated animals of all other groups except in rare instances in which fatty deposition in glomeruli and tubular epithelium was more prominent. Glomerular crescents, occasionally noted in salt-treated actively growing animals, were sometimes observed in treated birds with a reduced caloric intake, particularly in the few with more pronounced glomerular changes and the largest hearts.

Tubular atrophy was evident in the kidneys of birds given restricted diets. This was less marked in treated than in control animals and involved the convoluted tubules to a greater extent than the distal collecting portions of the nephron.

In order to indicate that some advancement in age when the animal is first exposed to salt combined with relatively mild restriction of food intake will fail to elicit all the renal changes observed in young chicks with a high salt intake but fed ad libitum, the following experiment is recorded:

Six week old birds were maintained on an ascending scale of salt, from 2 per cent to 9.1 per cent, but fed 60 Gm. of food daily. There were corresponding controls with the same food intake but without salt. The experiment lasted forty-five days. The average heart weight–body weight ratio of the 7 controls was 0.53, while that of the 8 treated animals was 0.73. The average renal weight–heart weight ratio was 0.63 for the controls and 0.76 for the experimental birds. Microscopically, the experimental kidneys revealed marked hypertrophy and tortuosity of blood vessels. The glomeruli, however, were not nearly as large or as fully tufted as those of treated birds fed ad libitum.



Photomicrographs of kidneys. Magnification, × 600. Arrows indicate arterial blood vessels.

Fig. 1.—Control chick aged 62 days. The bird had consumed 12 Gm. of food daily for thirty days.

Fig. 2.—Experimental chick aged 53 days. This bird had consumed 12 Gm. of food and ascending amounts of sodium chloride daily for thirty-one days. Note the visceral epithelial hypertrophy, the looped capillaries and the increase in reticulum of the glomerulus to the right. The artery to the left is more tortuous

(Legend continued on next page)

COMMENT

It was the purpose of this study to determine whether the characteristic cardiorenal changes observed in young chicks given a 3 per cent salt diet and fed ad libitum could be reproduced under a variety of nutritional states with variations in the concentration and daily levels of salt. It seems clear from the data given that such changes are obtained in their entirety only when there is rapid and unhampered growth. Nutritionally induced disturbances of growth or the natural decline of the rate of growth prior to maturity or its cessation after maturity interfere with their full evolution. Provided certain daily levels of salt are reached, which on the average must exceed 0.3 Gm. per hundred grams of body weight, neither the concentration of salt in the food nor a progressive increase in the amount ingested daily would seem to be essential factors. The concentration of salt must not, however, be so high as to interfere with the animal's consumption of food. The amount of water ingested, to which we ascribed considerable importance in a previous communication, can in no way be separated in its effects on the heart and the kidneys from that of the high salt intake. The two must perforce be intimately linked if water intoxication is to be avoided. With levels of salt above 0.3 Gm. per hundred grams of body weight, water consumption will automatically exceed 50 cc. per hundred grams of live weight of bird daily.

Cardiac enlargement was a constant finding common to all groups with a high salt intake. The degree of hypertrophy appeared to be unrelated to the amount of salt ingested daily, provided this exceeded the basic limit, and to the growth and nutritional status of the animal,

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and thicker walled than the control vessels in figure 1. This degree of glomerular change was exceptional for salt-treated birds on this diet.

Fig. 3.—Control chick aged 61 days. This bird was fed 23.5 Gm. of food daily for thirty-one days.

Fig. 4.—Experimental chick aged 70 days. The chick was given 23.5 Gm. of food and ascending amounts of sodium chloride daily for forty days. Note the absence of glomerular changes but the pronounced tortuosity and thickness of the preglomerular artery as compared with the control in figure 3.

Fig. 5.—Control chick aged 72 days. The bird was allowed to consume food ad libitum.

Fig. 6.—Experimental chick aged 72 days. This bird received food containing sodium chloride, 5 per cent, ad libitum for sixty-five days. Note the marked degree of glomerular hypertrophy with newly formed broad tufts or papillae. There is increased deposition of reticulum within the latter. There is also visceral epithelial hypertrophy. The tortuous hypertrophied blood vessels are clearly apparent.

Fig. 7.—Control bird aged 196 days, allowed food ad libitum.

Fig. 8.—Experimental bird aged 196 days. This bird had food ad libitum with ascending amounts of sodium chloride for thirty-eight days. It was an exception in presenting enlarged glomeruli of these dimensions. Note, however, their smooth character associated with increased size of the core and increase in number of nuclei but without tufting or papillary formation. The medial coats of the preglomerular arteries are evidently hypertrophied.

provided there was no profound loss in body weight. These hearts were 31 to 62 per cent heavier than their respective controls when weights were expressed per unit of body weight, and these values were highly significant statistically in all instances. When there was marked loss of body weight, 25 per cent or more over the experimental period, cardiac weight tended to be retained at the preexperimental level or to the extent of 16 to 18 per cent above that of similarly undernourished controls.

Since high salt intake is instrumental in elevating the mean blood pressure in birds, it may be pertinent to inquire whether cardiac hypertrophy was the result of this elevation. From our limited data, however, the increase in blood pressure was quite variable and seemed to be appreciable only when body weight was kept relatively stable in the young bird or when loss of weight was prevented in the adult animal. Since cardiac hypertrophy was a general phenomenon, it would seem unlikely that it was entirely due to hypertension. Other factors undoubtedly played an important role in its production.

The effect of high salt intake on the kidney was far more variable. Glomeruli, tubules and arterial blood vessels seemed to respond proportionately and as a unit only in chicks in which growth was unhampered. In all others, in which growth or nutrition was not in accord with this, these various elements responded independently if at all.

Glomerular hypertrophy was marked and constant only in rapidly growing birds. It failed to appear when loss of body weight was appreciable and was rarely pronounced and almost always symmetric in the other groups in which growth was either sharply restricted or inhibited or in well fed adults. The development of new glomerular tufts or papillae occurred only with unhampered growth. In view of these findings it is doubtful whether marked glomerular hypertrophy, particularly the new formation of tufts or papillae, can be regarded as a compensatory mechanism only. It is rather to be viewed as an exaggerated response to the stimulus of excess sodium chloride at a time when body growth is active.

Increase in tubular mass as represented by increase in renal weight was in most instances not significant statistically. Nevertheless, it seemed to follow that increase in renal weight varied directly with the increase in daily salt intake. It was, however, unrelated to glomerular or vascular changes.

Increase in tortuosity and medial hypertrophy of the interlobular arteries and their preglomerular arterioles in the inner third of the renal cortex was a constant finding. It varied in degree. It was most pronounced in salt-treated chicks fed ad libitum but was a promi-

nent feature in all other experimental groups except those with marked reduction of body weight. Here the integrity of the vessels was more apt to be maintained with respect to what they were prior to the experimental period, by contrast with the marked atrophy in the control animals. There was no strict correlation between these vascular changes and glomerular or tubular hypertrophy or hypertension. They seemed to be associated more closely with altered hemodynamics of the kidney.

SUMMARY AND CONCLUSIONS

Young chicks and older birds were divided into groups and fed either calorically restricted diets or food ad libitum. Within each group there were control and experimental animals. The latter were fed varying concentrations of sodium chloride with their food or, independent of the concentration, a fixed or an ascending amount of salt daily. The amount of sodium chloride ingested varied from 0.17 to 0.74 Gm. per hundred grams of body weight daily in different experimental groups and during different periods within certain of these groups. This did not include the amount of salt ordinarily present in the feed, which amounted to about 0.5 per cent.

In young chicks fed restricted diets the loss or gain in body weight in individual groups was respectively as follows: a loss of 30 to 36 per cent over two to three weeks; a loss of 23 to 27 per cent over two to four weeks; a loss of 4 to 7.5 per cent over three to four weeks; a gain of 25 to 50 per cent over four to six weeks. Young chicks allowed food ad libitum gained 85 to 117 per cent in body weight over a little more than a three week period.

The following conclusions are drawn from these experiments:

1. The daily load of sodium chloride apparently must exceed 0.3 Gm. per hundred grams of body weight if morphologic changes in heart and kidney are to take place. Water consumption, which under these circumstances will exceed 50 cc. per hundred grams of live weight of bird daily, cannot be dissociated in its effects from the salt load if water intoxication is to be avoided.

2. Cardiac hypertrophy is a constant finding in all experimental animals, independent of age, status of growth or amount of salt ingested above the basic limit. It is, however, largely relative and restricted to maintenance of the preexperimental weight of the heart when body weight has declined 25 per cent or more over the experimental period.

3. Renal hypertrophy, representing basically increase in tubular mass, is variable in its response to high salt intake. There are indications, however, that renal weight increases in direct proportion to the progressive increase of the daily load of sodium chloride.

4. Marked renal glomerular enlargement and the new formation of glomerular tufts or papillae occur only in experimental animals in which growth is rapid and unhampered. When growth is nutritionally restricted or when it is slowed or arrested as a result of natural aging processes, glomerular enlargement is at best limited, while newly formed tufts or papillae are practically never developed or are at most abortive.

5. Increased tortuosity and medial hypertrophy of renal arteries and preglomerular arterioles is a fairly constant finding in birds with a high salt intake. It is most markedly developed in animals in which growth is unhampered and is least pronounced in animals in which there has been profound loss in body weight.

6. The mean blood pressure is elevated in birds with a high salt intake, while the cardiac rate in young chicks is concomitantly diminished.

7. While the incidence of ascites is increased in calorically restricted animals with an elevated salt intake, hydropericardium is a frequent finding in all experimental animals, independent of the state of growth or nutrition.

8. Profuse intestinal hemorrhage occurs in birds on calorically restricted diets. It is as frequent in control as in experimental animals.

PIGMENT CHANGES IN EXPERIMENTAL WHOLE THICKNESS SKIN GRAFTS

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WHETHER pigment always invades the surrounding tissue after transplantation of skin has not been determined. On the one hand, Loeb,[1] Carnot and Deflandre[2] and Sale[3] showed that when 1 to 2 mm. pieces of black skin were transplanted to white areas the black pigment invaded the surrounding white area for a period of about ninety days. However, if similar white grafts were transplanted to pigmented areas, the pigment invaded the white graft, and later no trace of the graft was present. In 1932 Seevers and Spencer[4] reported that in the case of a pigmented rectangular graft with an area of about 2 sq. cm. there was no invasion of the surrounding area. Likewise, after a white graft of the same size was transplanted to a pigmented area, it was not invaded by the surrounding pigment during an observation period of nine months. In several cases the edges became slightly darker, but in no case was there actual pigmentary invasion. Seevers and Spencer also reported that the direction of the hair growth of the transplanted skin changed to conform to that of the surrounding skin.

Earlier work in this laboratory[5] with small grafts supported that of Loeb and others. It seemed possible, however, that migration of the pigment might not occur with larger grafts. For that reason I have carried out transplantations with large size grafts. This report concerns that work.

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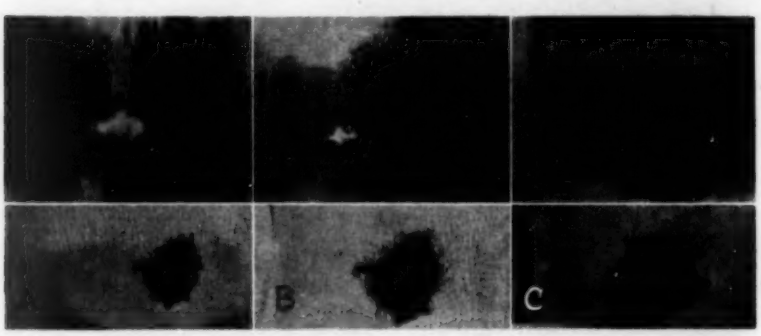
EXPERIMENTAL PROCEDURE

Black and white guinea pigs weighing 8 to 13 ounces (227 to 368.5 Gm.) were used. After clipping, the guinea pigs were shaved and washed, and the skin was sterilized with tincture of merthiolate. With a sterile cork borer 17 mm. in diameter, two circular areas of 2.27 sq. cm. were marked out, one in the black and the other in the white skin. The skin so outlined was excised and placed on a piece of gauze moistened with isotonic solution of sodium chloride. After hemostasis had been secured by pressure, the grafts were sutured into place, the black graft in the white area and vice versa. All of the grafts were placed at an angle of variance with the normal direction of the hair fibers. The completed grafts were covered with petrolatum gauze and a moist sea sponge wrapped in gauze, and this dressing was held in place by an adhesive strap. The grafts were undisturbed for twelve days; then the dressings and the sutures were removed, and the progress was noted. In this way, black and white grafts of the same age and subject to the same conditions were observed. Observations were made at intervals until the white grafts had disappeared completely.

RESULTS

In 11 of 50 guinea pigs takes of both the black and the white graft were obtained. These 11 animals are the only ones discussed here.



White and black skin grafts: *A*, forty days after transplantation. Note beginning invasion of the white graft. *B*, seventy days after transplantation. *C*, one hundred and two days after transplantation.

It was noted that during the first four weeks following transplantation the grafts shrank to about two thirds to one half of the original size. After the original shrinking stopped, the pigment of the black graft gradually invaded the surrounding white area. This extension of the black pigment continued for as long as ninety-three days. In direct contrast to this, in all 11 animals the white graft was gradually invaded by the surrounding black pigment until the graft was no longer visible. In 9 of the 11 guinea pigs this invasion of the white graft was completed within sixty to eighty days. However, in the remaining guinea pigs, 117 and 153, the process took one hundred and three and one hundred and twenty-nine days, respectively. These two retarded invasions are described in detail in the accompanying table.

The hair growth of the grafts was sparse, as many of the hair cells died during the transplantation. The hair grew out of the grafts in the original direction of growth. It was particularly interesting that in the case of the white grafts white hair continued to emerge from the grafted area for as long as five months, even though the original white graft had become completely pigmented.

COMMENT

The work presented here shows that when a large unpigmented skin graft is transplanted to a colored skin area the surrounding pigment gradually invades the graft. This result is in complete agreement with the earlier work of Loeb and others, who used smaller grafts, and is opposed to that of Seevers and Spencer. As Trotter and Dawson [6]

Detailed Presentation of Retarded Pigmentary Invasion of Two White Skin Grafts

Date of Observation	Black Graft, Mm.	White Graft, Mm.
Guinea Pig 117		
2/ 9 (day of operation)	17 × 17	17 × 17
3/18	12 × 15	12 × 8
3/27	15 × 18	10 × 7
4/ 5	17 × 18	9 × 6
4/17	20 × 22	6 × 4
4/27	21 × 23	3 × 2
5/10	22 × 23	0.5 × 0.5
5/19	22 × 23	0 × 0
Guinea Pig 153		
4/25 (day of operation)	17 × 17	17 × 17
5/19	5 × 5	10 × 7
5/23	5 × 5	10 × 7
6/ 5	10 × 9	4 × 7
6/18	15 × 12	1.5 × 2 and 1 × 1
6/24	15 × 12	0 × 0

have previously stated, the direction of the hair growth apparently remains constant, being unaffected by the movements and pressure of the surrounding hair growth.

SUMMARY

White skin grafted in place of colored skin is completely invaded by the surrounding pigment in sixty to one hundred and twenty-nine days. The hair color remains the same up to five months, even though the underlying grafted area becomes pigmented. The hair direction of the graft is not affected by external influences.

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6. Trotter, M., and Dawson, H. L.: Anat. Rec. **50**:193, 1931.

HOMOTRANSPLANTATION OF FETAL SKIN

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CLINICALLY, as early as 1884, Lucas [1] used skin from the prepuce of a newborn infant for homotransplantation with apparent success. This type of grafting was later revived by Eisenberg [2] and Ashley,[3] both of whom obtained results comparable with those of autotransplantation. Sabella [4] and Stern [5] reported success in using fetal membranes for the repair of burned or ulcerated surfaces. De Rotth [6] reported complete success in 8 cases of conjunctival defects repaired by the use of fetal membranes obtained at cesarean sections. Experimentally, Willis,[7] using skin from young rat embryos, reported excellent results with this tissue and suggested the use of materials taken from human embryos of the six to twelve week stage when sufficient skin is not available from the patient.

On the other hand, Lexer,[8] on the basis of his clinical observations, stated that although there was plainly an early enlargement of the epithelial islands following transplantation of skin of a fresh fetus, the entire flap was cast off in the third week. Coakley [9] reported that he and his co-workers were unable to get any better results with preputial skin than with adult skin. Experimentally, Skubisrewski [10] buried embryonic material subcutaneously in the hen and followed the result by repeated biopsy. He found that early multiplication and proliferation of the cells occurred but that this was later followed by regression and encapsulation of this material. Sartori [11] implanted embryonic material

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This work was done under a grant from the Institute of Medical Research, Christ Hospital, Cincinnati.

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in rabbits' eyes; he noted that, although there was early growth, after a certain stage all the implants succumbed to rapid involution and were completely resorbed.

It is apparent from these reports that the status of transplantation of fetal skin has not been definitely settled. An attempt has been made here to compare the results of homotransplantation of fetal and adult skin as accomplished by a method that yielded 80 to 90 per cent success in autotransplantation.

EXPERIMENTAL METHOD

Black and white guinea pigs weighing from 8 to 13 ounces (227 to 368.5 Gm.) were used as recipients. A 1 mm. square of black skin from each fetus and each adult was transplanted to a white rump area by the skin flap procedure described previously.[12] The fetal skin was obtained from fetuses 1 to 7 cm. in length (twenty-five days to term) immediately after cesarean section; pure black mothers were used. Pentobarbital sodium was the anesthetic. For the transplantation of adult skin, guinea pigs 4 to 5 months of age were used.

The term "initial takes" as used in this paper refers to the grafts which survived the early period following transplantation and were adherent and apparently well nourished when the flap was removed. In guinea pigs 19 and 126 the flap was accidentally disturbed earlier, but the grafts, even though exposed, progressed as described.

The survival time of the graft, given in days, refers to the period from the time of transplantation of the skin until the black graft was no longer visible.

RESULTS

For convenience the experiments have been placed in the following groups: group 1 (fetuses 1 cm. in length), 16 grafts; group 2 (2.5 cm. fetuses), 18 grafts; group 3 (4 cm. fetuses), 58 grafts; group 4 (4.5 cm. fetuses), 52 grafts; group 5 (5 cm. fetuses), 22 grafts; group 6 (6 cm. fetuses), 24 grafts; group 7 (adult tissue), 66 grafts. In the 16 experiments in group 1 (1 cm. fetuses) there were no takes. This was true also of the grafts in group 2 (2.5 cm. fetuses).

Eight of the 58 grafts in group 3 (4 cm. fetuses) showed initial takes. In this group most of the grafts remained the same size until their disappearance thirty-two to fifty-nine days after operation. An exception to this was guinea pig 84, whose graft increased from its original size of 0.1 by 0.1 mm. to 3 by 2 mm. by the thirtieth day but then seemed to melt away and was gone two days later.

Four of the 52 grafts from group 4 (4.5 cm. fetuses) were successful. Of these the graft on guinea pig 19 had increased to 4 by 2 mm. nineteen days after operation; it then gradually became smaller and disappeared fifty days after transplantation. The graft on guinea pig 126 had increased to 5 by 1.5 mm. nineteen days after operation, but disappeared nine days later.

Six of the 22 grafts in group 5 (5 cm. fetuses) were initial takes and grew for a short period. The graft on guinea pig 11 had increased to 10 by 3 mm. after operation, but gradually began to regress and disappeared in twenty-seven days.

Only 1 of the 24 grafts in group 6 (6 cm. fetuses) showed an initial take. In this animal the graft remained 2 by 1 mm. for forty-five days after transplanta-

12. Barker, D. E.: Arch. Path. **32**:425, 1941.

tion, then began to fade, and was no longer visible sixty-five days after the operation.

Of the adult series (group 7) of 66 experiments, 14 (21 per cent) were successful at first. In 12 of these the graft remained the same size for a period of twenty-one to twenty-four days and then gradually became smaller until it disappeared. The graft on guinea pig 60, however, had increased to 3 by 2 mm. by twenty-seven days after transplantation. Seven days later it was noted that the graft was beginning to fade, and it was no longer visible forty-five days after transplantation. The graft on guinea pig 56 increased to 3 by 1 mm. and lasted for forty-one days after operation.

COMMENT

In this series of experiments in which skin grafts from guinea pig fetuses of 1 to 7 cm. in length were used, apparently the younger is not as well prepared to withstand transplantation as is the older fetal skin (see table). The percentage of primary takes when the older fetal tissues were used is about comparable with that recorded when adult tissues were used. The main difference between the use of older fetal

Transplantation of Adult and Fetal Skin

Group	Length of Fetus, Cm.	Experiments	Initial Takes	Percentage of Initial Takes	Percentage of Permanent Takes
Fetal tissue 1	1	16	0	0	0
2	2.5	18	0	0	0
3	4	58	8	13	0
4	4.5	52	4	7.7	0
5	5	22	6	27	0
6	6	24	1	4	0
Adult tissue	..	66	14	21	0

tissues and that of adult tissues is that with the fetal tissues there are early proliferation and multiplication of the cells with enlargement of the graft, while, on the whole, the adult grafts do not increase in size. The fetal grafts usually disappear by a slow decrease in size. Loeb [13] showed that with homotransplantation of adult tissues there is gradually a decrease in the number of mitotic figures present following transplantation until the seventh day, when all have apparently gone. In the use of fetal tissues this mitosis apparently persists up to about the twenty-fifth day, when regression sets in. The results given here are in agreement with the works of Lexer,[8] Skubisrewski [10] and Sartori.[11]

CONCLUSIONS

In the guinea pig the use of fetal skin tissues for permanent transplantation is apparently no more successful than that of adult tissues.

The early proliferation of the fetal skin is of short duration and is followed by either a rapid melting away of the graft or a slow regression.

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COMMON TRUNK OF PULMONARY VEINS TRIBUTARY TO THE PORTAL VEIN, WITH A MULTIPLE CARDIAC ANOMALY

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A PULMONARY venous system draining in whole or in part into the portal vein is rare. In 1942 Brody [1] collected 102 cases in which part or all of the pulmonary venous system drained into the right side of the heart or its tributaries and described 4 additional cases. Two years later, Hughes and Rumore [2] reported 2 instances and collected 7 cases, not mentioned by Brody, from the literature. However, they did not include the case reported by Conn and others,[3] who in turn referred to the cases of Barge and van Oijen [4] and Goltman and Stern,[5] which are not tabulated elsewhere. In a recent paper on the surgical significance of anomalies of the pulmonary veins, Brantigan [6] referred to 7 cases more recently reported and added 2 cases (in one the anomaly was observed by him at operation, and in the other it was visualized through a thoracoscope). Among the 127 cases there were only 5 (all cited by Brody) in which all or part of the pulmonary venous system drained into the portal vein. The only case of association of a cardiac anomaly and a pulmonary venous system draining completely into the portal vein was reported by Arnold,[7] in 1868. An additional case of this type is recorded in the present paper. The anomalies occurred in a stillborn infant and were associated with additional developmental defects.

REPORT OF A CASE

A premature white infant was born dead during the estimated sixth month of pregnancy. The onset of labor followed a long bus ride taken by the mother. The

From the Department of Pathology, University of Oklahoma School of Medicince and University Hospitals, Oklahoma City.

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3. Conn, L. C.; Calder, J.; MacGregor, J. W., and Shaner, R. F.: Anat. Rec. **83**:335, 1942.
4. Barge, J. A., and van Oijen: Ztschr. f. Anat. u. Entwcklngsgesch. **98**: 760, 1932; cited by Conn and others.[3]
5. Goltman, D. W., and Stern, N. S.: Am. Heart J. **18**:176, 1939.
6. Brantigan, O. C.: Surg., Gynec. & Obst. **84**:653, 1947.
7. Arnold, J.: Virchows Arch. f. path. Anat. **42**:449, 1868.

presentation was a double footling. The mother, aged 25 years, had 3 children, who were delivered uneventfully at term. During the third pregnancy, two years previously, a diagnosis of syphilis was made, and treatment was carried on during the pregnancy and subsequently. On this admission, the Mazzini test of the blood was negative; a test for the Rh factor was positive.

At necropsy the fetus was well developed and well proportioned; it was 31 cm. long and weighed 605 Gm. There were no anomalies of the mouth. The neck, chest, abdomen, external genitalia and back were as usual. The upper and lower extremities were proportionate and symmetric.

Ten centimeters of moist cord was attached to the umbilicus. The umbilical vein and remains of the urachus appeared as usual. The right umbilical artery was absent; the left measured 0.3 cm. in diameter. The cecum was mobile and in the right upper quadrant of the abdomen. A 2 cm. segment of colon, composed of the distal ascending and proximal transverse portions, did not have a mesentery. The spleen was absent. The liver was located on the right, with the lobes of the usual proportions. The stomach and the pancreas were in the right upper quadrant of the abdomen. The first portion of the duodenum and the head of the pancreas curved posteriorly on themselves. The distal portions of the duodenum were in their usual locations.

There were no ossification centers in the sternum. The costochondral junctions were straight. The thymus gland was bilobed and measured 1.3 by 0.7 by 0.5 cm.; from the upper margin of each lobe a finger-like projection extended into the root of the neck for a distance of 0.8 cm. The pleural cavities contained no excess fluid; their surfaces were smooth and glistening.

The pericardial cavity contained no excess fluid; its surfaces were smooth and glistening. The heart measured 2.5 cm. from base to apex and 2.5 cm. across the base. The apex pointed slightly to the right and was made up entirely of the left ventricle. The aorta arose from the diminutive right ventricle (fig. 1*A*). The pulmonary artery seemed to arise from the left ventricle. There was only one atrium. The single atrioventricular orifice had a bicuspid valve and opened into the left ventricle. No pulmonary orifice was present. An interventricular septal defect, 0.7 cm. in diameter, was present in the superior portion of the septum (fig. 1 *B*). The atrium did not communicate with the right ventricle. The aortic orifice was 0.9 cm. in circumference; its valves were delicate. The orifices of two coronary arteries were present in the left sinus; the orifice of one, in the right.

The innominate and left common carotid arteries arose from the arch of the aorta as a single trunk. The ductus arteriosus was patent, easily admitting a probe. The descending portion of the thoracic aorta and the abdominal aorta were on the left.

The tributaries of the superior vena cava were as usual, and the vein itself entered the right side of the atrium. Inferiorly the atrium received two venous trunks: a right hepatic vein on the right and a venous trunk continuous with the inferior vena cava on the left (fig. 2). A communicating branch connected the two trunks just before they entered into the atrium. The inferior vena cava was located on the right, inferior to the renal veins. Superior to the renal veins there was a single venous trunk on the left, formed by the persistence of the prerenal segment of the left subcardinal vein and the proximal portion of the left vitelline vein. Before entering the atrium, this vein received a left hepatic vein and the ductus venosus.

The left and right pulmonary veins united in the midline, posterior to the heart, and the single trunk thus formed descended to pass through the diaphragm, anterior

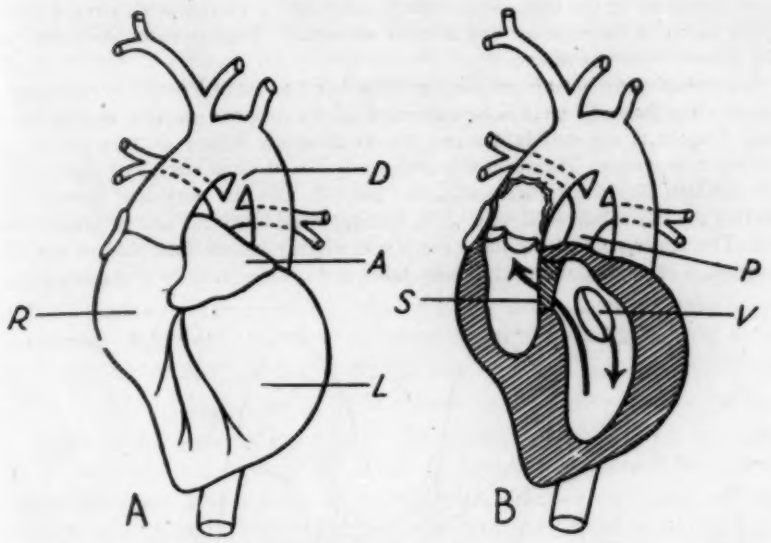

Fig. 1.—*A*, anterior view of the heart: *D*, patent ductus arteriosus; *A*, single atrium; *R*, right ventricle; *L*, left ventricle.

B, anterior view of heart, exposing the chambers and orifices: *P*, pulmonary artery with atretic orifice; *V*, single atrioventricular valve; *S*, interventricular septal defect.

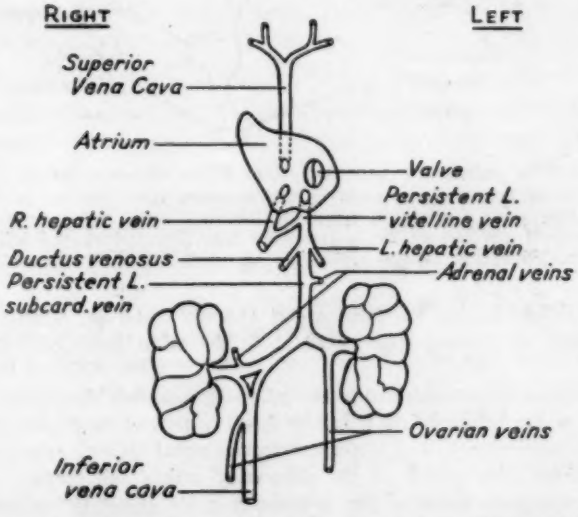

Fig. 2.—The atrium and its tributaries. The anomalous position of the vessels inferior to the atrium is shown in their relation to the kidneys.

to the esophagus (fig. 3). The common trunk received a tributary vein from the lesser curvature of the stomach (coronary vein) before uniting with a trunk formed by the union of the superior and inferior mesenteric veins to form the portal vein. The splenic vein was absent.

The lungs were not air containing. The left had three lobes; the right had an incompletely formed fourth lobe composed of the superior portion of the inferior lobe. The liver, the gallbladder and the extrahepatic biliary ducts were of usual size and appearance. The adrenal glands were also of usual size. The right adrenal vein emptied into the right renal vein; the left, into the persistent prerenal segment of the left subcardinal vein. The kidneys were lobulated and of proportionate size. The upper pole of the left kidney was slightly higher than that of the right, as usual. The vagina, uterus, fallopian tubes and ovaries were of proportionate size.

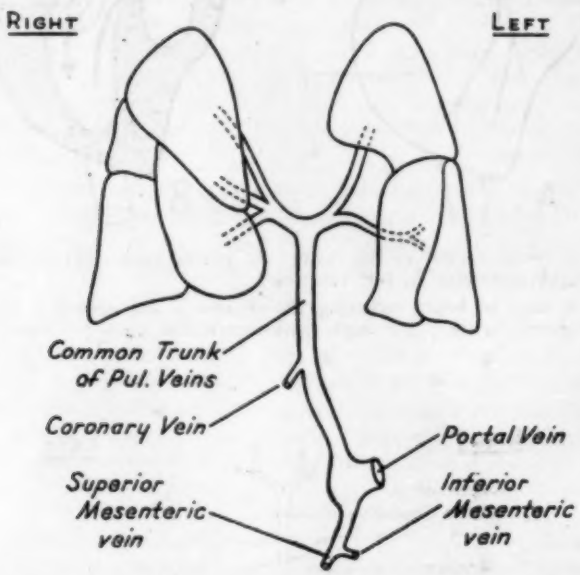

Fig. 3.—The pulmonary venous system. The common trunk formed by the union of the pulmonary veins receives the coronary vein, inferior to the diaphragm, before uniting with a common trunk of the mesenteric veins to form the portal vein. The first portion of the portal vein was directed to the left as indicated, although the liver was on the right side as usual.

In the cranial cavity, the falx cerebri and the tentorium cerebelli were intact. No anomalies or changes were noted about the brain, its vessels and supporting structures.

The findings are summarized in the following anatomic diagnosis: prematurity; atelectasis of the lungs, fetal; trilocular heart with two ventricles, absence of the right atrioventricular orifice, interventricular septal defect, transposition of the arterial trunks with atresia of the pulmonary orifice and patency of the ductus arteriosus; common trunk of the pulmonary veins tributary to the portal vein; right hepatic vein entering into the atrium; persistence of the proximal left vitelline vein and of the prerenal segment of the left subcardinal vein with atresia of the prerenal segment of the right subcardinal vein; atresia of the right umbilical artery; situs inversus of the stomach and the pancreas with aplasia of the spleen.

COMMENT

In an early stage of embryonic development, an anastomosing network of capillaries comprises the circulatory system.[8] As the embryo enlarges, parts of this system differentiate to form the heart, the arteries and the veins. Concomitantly, certain of the channels enlarge and others disappear as the various vascular systems develop. At one stage the umbilical, vitelline, splanchnic and cardinal venous systems converge in the septum transversum and anastomose with one another. Most of the communicating vessels disappear. Occasionally, some remain.

Persistence of one or more of the primitive connections between the pulmonary and splanchnic or vitelline systems accounts for one group of anomalies observed. In such an instance the fully developed pulmonary venous system partially drains into any of the tributaries of the right atrium derived from the cardinal system or into the portal vein, which is derived entirely from the vitelline system. All or part of the pulmonary veins may be diverted into the right atrium when the heart develops abnormally or when the primitive pulmonary vein connects at an unusual site. If the primitive pulmonary vein becomes atretic or fails to connect with the heart, one or more of the channels communicating with the splanchnic or the vitelline plexuses remain patent. Thus, all the blood returning from the lungs would empty into one or more of the tributaries of the right atrium or into the portal vein, as occurred in the case herein presented.

The circulatory system in this case was in effect that of a two-chambered heart (fig. 4). All venous blood returned to the single atrium. From there it reached the left ventricle, which did not communicate with the pulmonary artery. The left ventricle pumped blood through the interventricular septal defect and the right ventricle into the aorta. The patent ductus arteriosus allowed blood to enter the pulmonary arteries.

The initial defect of development was probably the failure of the primitive pulmonary venous system to drain into the heart. Since, under these circumstances, it was impossible to segregate the oxygenated and unoxygenated blood, this initial defect might have been the cause of the failure of the heart to develop further.

This type of circulatory system is apparently compatible with life only on a limited basis. In the 3 previous cases in which the total pulmonary venous system drained into the portal vein, the duration of life was fifteen weeks,[7] fifteen days [9] and three months,[10] respectively. In

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9. Ghon, A.: Beitr. z. path. Anat. u. z. allg. Path. **62**:175, 1916; cited by Brody.[1]

10. Munck: Acta path. et microbiol. Scandinav. **10**:321, 1933; cited by Brody.[1]

the case herein reported, the infant might have lived as long as the ductus arteriosus remained sufficiently patent to allow adequate circulation to the lungs.

The cardiac anomaly in Arnold's [7] case was in certain respects similar to the one reported here. The heart was in effect a two-chambered system, as a wide communication was present between the atriums and there was only a single ventricle. There was also atresia of the pulmonary orifice with patency of the ductus arteriosus. In addition, the spleen was absent.

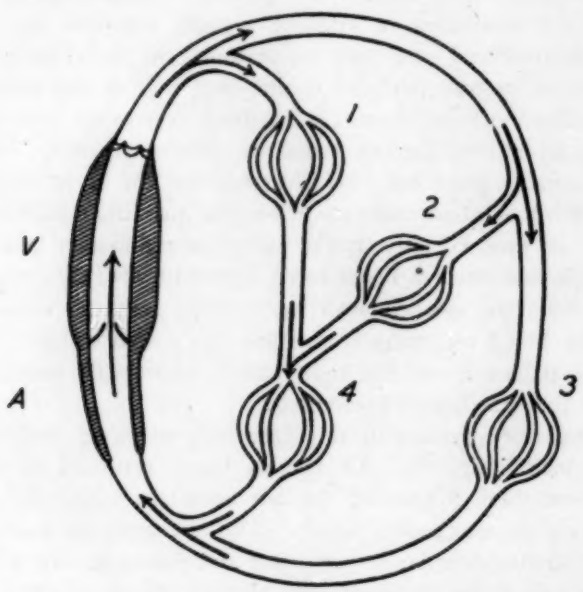

Fig. 4.—Diagram of the circulatory system: *A*, atrium; *V*, ventricle; *1*, pulmonary circulation; *2*, mesenteric circulation; *3*, systemic circulation; *4*, portal circulation.

Another unusual finding deserves comment. It is believed that the leftward rotation of the stomach is due to the mechanical influence of the rapid growth of the liver on the right side. In my case both the stomach and the liver were on the right side. It seems likely that the presence of the common trunk of the pulmonary veins inferior to the diaphragm may have interfered with the usual rotation of the stomach.

SUMMARY

A pulmonary venous system draining completely into the portal system was observed in a premature, stillborn girl, associated with a multiple cardiac anomaly and other developmental defects. This case

is believed to be the sixth reported instance of a pulmonary venous system draining in whole or in part into the portal vein and the fourth case in which such anomalous drainage was complete. It is apparently the second instance in which complete drainage was associated with a cardiac anomaly and the first case in which it was associated with this particular cardiac anomaly.

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STUDIES IN DYSTROPHIA MYOTONICA

VII. Autopsy Observations in Five Cases

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DURING the course of investigations of a group of patients with dystrophia myotonica at the Colorado General Hospital, 3 of the patients died, and autopsies were made. In comparing the sections from these autopsies with sections from autopsies made in 2 cases in which primary muscular atrophy was diagnosed, one of which was reported by Moleen, Johnson and Dixon [1] in 1932, we were struck by the similarity. A review of the histories obtained in these 2 cases convinced us that the patients had dystrophia myotonica instead of primary muscular atrophy. The pathologic changes found in these 5 patients are presented in this report. The heredity,[2] clinical features [3] and psychiatric aspects [4] of dystrophia myotonica have been discussed in previous articles of this series. Experimental investigations of myotonia,[5] creatine and creatinine excretion [6] and sugar tolerance [7] have also been reported.

REPORT OF CASES

CASE 1.—F. B., a white man aged 44 years, was first seen in April 1938, complaining of weakness of the hands and legs of many years' duration and recent hemoptysis.

The patient's father had died at about 75 years of age in a state hospital to which he had been committed with a diagnosis of senile dementia; his vision had been poor for a number of years before his death. The patient's mother was alive at 81 years but mildly diabetic. (She died shortly after the patient's death.) The

From the Departments of Pathology and Medicine, University of Colorado School of Medicine.

1. Moleen, G. A.; Johnson, W. C., and Dixon, H. H.: Arch. Neurol. & Psychiat. **27**:645, 1932.
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3. Waring, J. J.; Ravin, A., and Walker, C. E.: Arch. Int. Med. **65**:763, 1940.
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patient was the ninth of thirteen children. An older sister died in her fifties of cerebral embolism and bronchopneumonia. She was apparently simple minded, allegedly as a result of an injury to the head in childhood. Another sister, aged 56, was mildly diabetic. One sister died at about 44 years of age of "heart trouble." She was ill the last twenty years of her life and had frequently said that her hands stuck to things. Her husband said that often, on the first bite, her jaw would stay clamped for a while and that her jaw dislocated easily. An older brother, about 51 years old, was thin, walked with a "steppage" gait and was said by one of his brothers to look just like the patient; he wore glasses and had poor vision. One sister died at 16 months of age of diphtheria. Two younger brothers and two younger sisters apparently were normal.

The past history revealed that the patient had had mumps with no associated orchitis. He had typhoid fever at the age of 14 and pneumonia at the age of 22. At the age of 30 he was in the hospital with a pain in the right side which was attributed to a ureteral stricture. He had gonorrhea at the age of 22 and again at that of 33; following the second attack he had some joint pains and swelling. He went through the eighth grade and then did tire repair work and odd jobs. He married at the age of 22 and was divorced twelve years later. His wife had no children, although he said she was pregnant on several occasions and induced abortions.

At the age of 16 or 17 the patient first noticed that when he gripped an object strongly he had difficulty releasing it. At 22 to 23 years of age he realized that his grip was not as strong as that of his fellow workers. The difficulty in relaxing his grasp and the loss of strength in his grip gradually progressed. The strength in his arms and shoulders had also decreased. Two years before coming to the clinic he had been told that he walked as though he had a "wooden leg or was paralyzed." Since that time he had been conscious of increasing difficulty in walking. If while standing he became too interested in what he was doing, he was apt to lose his balance and have to take a step to regain it. During the last year he had noted stiffness of his ankles on starting to walk. As far back as he could remember his hands and feet had been cold. In the last six months his voice had become lower in pitch, less clear and somewhat husky. Occasionally he had difficulty in drinking water from a fountain. Print blurred when he read, and his eyes frequently burned and watered.

About six weeks before being examined the patient began to have some pain in the right costal region, which was worse on deep inspiration. The pain was associated with some cough and expectoration of a little mucoid material. After a week the pain went to his left side, and he coughed up some bloody sputum. The pain lasted about three weeks. He believed that he had had some fever with the onset of the pain. He was not short of breath and had noticed no edema.

The patient was a tall, well nourished, middle-aged white man. He was intelligent and cooperative. His voice was definitely nasal and his gait of the "steppage" type. He was bald with a graying rim of hair.

There was slight marginal blepharitis and chronic conjunctivitis. Slit lamp examination after use of homatropine hydrobromide showed many fine opacities, varying in size from that of dust particles to almost a millimeter in diameter, scattered throughout the cortex of the lens and extending well into the central area. They were grayish white but refractile and appeared blue and blue-green at times. The fetal nucleus was not involved. Vision with glasses was 20/20 in both eyes.

Most of the teeth were present, although in poor condition. The thyroid gland was small but firm. The lungs were normal to percussion and auscultation. The heart was normal in size. The heart sounds were somewhat faint but normal in

character. The aortic second and the pulmonic second sound were of about equal intensity. No murmurs were heard. Peripheral sclerosis was not evident. The blood pressure was 96 systolic and 64 diastolic in the recumbent position and 104 systolic and 72 diastolic in the sitting position. The systolic pressure fell about 10 mm. with deep inspiration.

Abdominal examination gave negative results. Both testes were possibly somewhat softer and smaller than normal.

Examination of the neuromuscular system showed the following variations from normal: The temporal muscles were markedly atrophied and the masseter muscles somewhat atrophied. The periorbital and perioral muscles were weak. The sternocleidomastoid muscles were almost completely atrophied, and when the patient rose from a reclining position his head fell backward and had to be supported by his hand. The muscles of the supraclavicular fossae and the deltoid muscles were somewhat atrophied. The biceps and especially the triceps muscle on each side were weak and somewhat atrophied. Both the flexor and the extensor muscles in the forearm were weak and atrophic. The muscles of the thenar and hypothenar eminences were only slightly involved but the interossei muscles were definitely atrophied. Flexion and extension of the thigh were fairly good, as was extension of the knees. Flexion of the knees was weakened. Plantar flexion of the feet was fair on the left and weak on the right. Dorsiflexion of the feet was markedly decreased.

The patellar and the abdominal reflexes were present and normal. The biceps and achilles reflexes could not be obtained. Chvostek's sign was positive. Trousseau's sign was negative.

Voluntary myotonia (persistence of the contraction of a muscle after cessation of voluntary effort at contraction) was evident in the movements of the fingers, thumbs and ankle. When the patient flexed his fingers, they could be extended only very slowly in spite of marked effort. The determined effort to overcome the persistent contraction in flexor muscles resulted in a rather characteristic distortion of the upper extremities: The elbows and the wrists were flexed to facilitate the extension of the fingers, and the wrist was gradually extended as the fingers slowly became extended—the small finger usually being the first to be extended completely and the index finger the last. The second time that the flexor muscles were contracted, extension occurred more rapidly, although still more slowly than normal. With each repetition of the movement the extension became more rapid until it occurred with normal rapidity. After a period of rest the difficulty returned.

The mechanical irritability of the muscles was generally increased; that is, they contracted more readily when struck with a percussion hammer than normal muscles. Many of the muscles, furthermore, on contracting remained contracted for many seconds (mechanical myotonia), forming a furrow or dimple, and then slowly relaxed. This was seen in the tongue, in the chin, in the deltoids, biceps and triceps muscles on each side, the flexor and extensor muscles of the forearm, the muscles of the thenar and hypothenar eminences, the quadriceps muscle on each side, and the left gastrocnemius muscle.

The Wassermann and Eagle tests of the blood were normal. The spinal fluid was normal. Roentgenologic examination of the chest on April 21 revealed no abnormalities of the heart or the lungs. The gastrointestinal tract was normal except for marked spasticity and stasis in the colon. On April 25 the blood serum calcium was 9.3 mg. and the serum inorganic phosphorus 2.8 mg. per hundred cubic centimeters. The blood cholesterol was 148 mg. per hundred cubic centimeters. The basal metabolic rate was —26 per cent. The electrocardiogram was

normal except for a somewhat high take-off of the S-T segment in lead I (1 mm.) and in lead II (1 mm.).

Course.—Injections of 25 mg. of testosterone propionate were started at the rate of two a week. On May 5 the patient again began to have a severe pain in the right costal region and coughed up some bloody sputum. He was admitted to the hospital on May 6 with these complaints and a temperature of 100 F. At about 4 a. m. the following day the patient suddenly became cyanotic and comatose. Respirations dropped to 3 or 4 per minute, and the trachea and lungs were full of moisture. In an oxygen tent and with stimulants, the patient improved. A roentgenogram of the chest revealed a homogeneous density of the right lower lobe and some increase of density of the left lower lobe. Sputum culture revealed the presence of Pneumococcus, type III. The patient was then treated with sulfanilamide, but the course was downhill and he died on May 10. The observations at autopsy are recorded in a subsequent section.

The clinical picture of this patient to the time of his last illness is typical of dystrophia myotonica. The features of the disease which he showed are: (1) the heredity characteristic of patients with the disease, (2) myotonia, (3) muscle atrophy, (4) cataracts, (5) baldness and a (6) low basal metabolic rate. The family history is in many ways characteristic of that usually obtained, in that several siblings were involved and the parents were stated not to have had the disease. In a previous article of this series [2] it was concluded that the disease is probably transmitted as a dominant characteristic but that it tends to become more severe and to set in at an earlier age in each succeeding generation. In the affected parent of this patient, the onset of the disease was probably late in life, and those changes which occurred before death were not of sufficient degree to be distinguished from the expected senile changes. This, we believe, is the reason why the disease, although transmitted as a dominant character, is frequently not recognized in the parents.

The distribution of the myotonia—that is, in the hand grasps and in the legs—is characteristic of the disease. Occasionally, as in the patient's sister, the myotonia is also seen in some of the muscles of mastication; rarely it may be widespread. The presence of myotonia is of great value in distinguishing these patients from patients with other types of muscular atrophy.

The tendency of the muscle atrophy of dystrophia myotonica to involve certain muscles early is well shown in this man. The regions usually involved include the muscles of the forearm, the sternocleidomastoid muscles, the dorsiflexors of the feet, the facial muscles and the quadriceps muscles. As the disease progresses, the atrophy becomes more general. The atrophy and the weakness are the main causes of disability and usually the motive of the patients' seeking medical advice.

Presenile cataract, if looked for with a slit lamp, can be found in most patients with advanced muscle atrophy. Early stages of the cataract may at times be present before the muscle changes.

Although myotonia, muscle atrophy and cataract are the triad by which the disease should be remembered, other dystrophic changes and endocrine changes occur frequently. The baldness, the low basal metabolic rate and the hypotension are evidences in this patient of some of these changes. Testicular atrophy is also commonly seen. Menstrual irregularities, infertility and enlargement of the thyroid gland may also be seen.

Case 2.—O. M., a 43 year old man, first came to the clinic in December 1935, complaining of poor vision. Two sisters, a brother and a nephew were also considered as having dystrophia myotonica. His father was operated on for cataract at about 60 years of age; a sister of his father was operated on for cataract at 65 years of age; a half-sister of his mother was thought to have had "locomotor ataxia," because she suffered from progressive inability to use her lower extremities from about the age of 30 years to her death at 64 years.

The patient was born in Illinois in 1895. He finished the eighth grade at the age of 16 years and then worked as a farmer and as a carpenter. He enlisted in the Army in 1917 and was discharged in 1919. He had measles, mumps and whooping cough in childhood and pneumonia and pleurisy in 1930. He was married in 1924 and had no children.

He wore glasses from the age of 12. At the age of 30 he was told that he had incipient cataracts. A few years later his vision began to fail rapidly, and at 40 years of age he was almost blind. Examination at that time revealed almost mature cataracts of both eyes. Slit slamp examination showed an almost complete opacity of each lens, with highly refractile globular opacities, which cast bluish reflections. In January 1936 a cataract was removed from the left eye, and in February 1936 one was removed from the right eye. For many years he had had some irritation of his eyelids and a slight discharge from his eyes.

In 1922, at the age of 27, he noticed that he had a poor grip, and in 1928 he noticed that if he grasped an object he could not immediately release it. The weakness of his hand grasp gradually increased; the stiffness did not change much. On beginning movement after rest the muscles of the lower extremities felt stiff. The stiffness in his hands and legs was worse in cold weather.

For twelve to fourteen years before being seen the patient had been very intolerant to cold, and his hands and feet easily became cold. During the same time he lost both strength and energy. In October 1933 he was found to have a basal metabolic rate of —37 per cent. Thyroid U. S. P. was administered, and the basal metabolic rate increased to normal with a dose of 10 grains (0.65 Gm.) one day alternated with 5 grains (0.32 Gm.) the next day. With this treatment the patient's sluggishness and malaise were improved. When seen in December 1935 he was still taking thyroid but did not think he was greatly benefited. He felt that there had been no change in the stiffness of his hands.

Examination in January 1938 revealed a well nourished, rather tall, fairly well developed white man of 43 years of age, with an expressionless "hatchet facies." He talked rather slowly. The gait was slightly but definitely of the "steppage" type, with some "slapping." The lens of each eye had been removed. The thyroid gland was easily palpable, being enlarged about one and a half times. The lungs and the abdomen were normal. The testicles were somewhat smaller and softer than normal.

A roentgenogram revealed the heart and the aorta to be of normal size and shape. The heart sounds were faint. The blood pressure was 100 systolic and 68

diastolic, and the pulse rate was 56 per minute. The peripheral vessels showed little if any evidence of sclerosis.

Examination of the neuromuscular system revealed that the patient had the typical myopathic facies as a result of atrophy and weakness of the muscles of expression. The temporal muscles were moderately atrophied, but the masseter muscles appeared in good condition. The sternocleidomastoid muscles were markedly atrophied. The muscles of the forearm were moderately atrophied. The hand grasp was weak, more so on the right, although the patient was right handed. The extensor muscles of the forearm appeared more affected than the flexors. Flexion of the thigh and leg was somewhat weakened. Dorsiflexion of the feet was weak, but definite atrophy of the dorsiflexor muscles was questionable. Active myotonia was present to a marked degree in the hand grasp and to a much less degree in the movements of the toes and ankles. The mechanical irritability of the muscles was increased, and mechanical myotonia was evident in the muscles of the chin, the extensor muscles of the wrist and fingers, the tongue, the deltoid muscles and the muscles of the thenar and hypothenar eminences.

The Wassermann test and the Eagle flocculation test of the blood were negative. The urine was normal. The blood counts showed no persisting abnormality. A roentgenogram of the skull in January 1936 showed the sella turcica normal and the pineal body, partially calcified, in normal position. The basal metabolic rate on Oct. 15, 1933, was —37 per cent, and the cholesterol of the blood at the same time was 180 mg. per hundred cubic centimeters. While the patient was taking thyroid, the basal metabolic rate varied from —26 to plus 8 per cent. In December 1935 three determinations were made of the calcium content of the blood, showing 11.0, 10.1 and 11.2 mg. per hundred cubic centimeters; the associated phosphorus values were 3.8, 3.8 and 3.3 mg. per hundred cubic centimeters.

In December 1940 the patient fell down a flight of steps and fractured his skull. He died in ten hours without recovering consciousness.

Case 3.—J. B., when first seen, in 1937, at the age of 58, stated that he was very well until about the age of 46 years when, during a game of tennis, he noticed a weakness of the right hand. The weakness of the right hand gradually progressed, and atrophy of the muscles of the forearms became evident. At about 52 years of age he noticed atrophy in his left forearm. Later his legs became increasingly stiff and weak. The stiffness was worse after he had been sitting for some time and in cold weather. It diminished after walking. He stubbed his toes when he walked. His articulation had been poor for three to four years.

For several years the patient had had marked generalized weakness. His hands became cold easily. He had lost about 5 pounds (2.5 Kg.) in the last year, and when seen weighed about 100 pounds (45.4 Kg.). His best weight was 134 pounds (60.8 Kg.) at the age of 20 years.

He had been wearing glasses for more than fifteen years. During the last five years, his eyes had been watering.

He had measles and whooping cough in childhood, a febrile disease ("typhoid-malaria") at 19 years and influenza at 41 and 44 years of age. He was born in Texas, one of twins. The twin brother died at 8 months of age. An older brother had the same disease, as did his son and daughter.

When examined in September 1937 the patient appeared somewhat older than his stated age of 58 years. He was of medium height and of rather slight build, cooperative and intelligent. He talked in a nasal, monotonous, low-pitched voice, at times difficult to understand. He walked with a definite "steppage" gait, with

the body flexed forward. The skin over the face was tight, thin and shiny. He was bald, and his teeth had been extracted. On examination the thyroid gland was not enlarged; the lungs and the abdomen were normal, and the testicles were definitely smaller and softer than normal.

The eyes showed chronic conjunctivitis. The cornea, the anterior chamber and the iris of each eye were normal. The lens of each eye showed early subcapsular and posterior starlike opacities. Numerous small punctate opacities of various sizes occurred throughout the entire lens but were more numerous under the anterior and posterior capsules. With a slightly minus lens, the vision was normal.

The heart and the aorta were of normal size and shape as observed in the roentgenogram. At the apex the first sound was of moderate intensity and was followed by a rather rough, fairly loud, high-pitched systolic murmur. The blood pressure was 104 systolic and 70 diastolic. The electrocardiogram was normal.

As to the neuromuscular system, the temporal muscles were markedly atrophic; the masseter muscles, less so. The periorbital muscles, the muscles of the cheeks and the orbicularis oris muscle were weak and atrophic, producing the typical myopathic facies. Only a few fibers of the sternocleidomastoid muscles were left. All the muscles of the shoulder girdle and of the trunk showed moderate atrophy. The triceps and biceps muscles and the muscles of the forearms and hands on each side were markedly atrophied. Flexion and extension of the thigh were fairly good. Flexion and extension of the legs were weak. Dorsiflexion of the feet was very weak. All the muscles of the lower extremities were somewhat atrophic. Voluntary myotonia was present in the abductor muscles of the thumbs. All the muscles showed a somewhat increased mechanical irritability. Mechanical myotonia was present in the chin muscles, the tongue, the deltoid muscles, the extensor muscles of the fingers and wrists, the thenar and hypothenar muscles, the gluteal muscles and the calf muscles. Electrical testing revealed a myotonic reaction, modified by the atrophy. The patellar reflexes were present. The achilles tendon reflex could not be obtained. Chvostek's and Trousseau's signs were lacking.

The Wassermann and the Eagle test of the blood were negative. The urine and the blood were normal. The basal metabolic rate in June 1937 was —19 per cent. On June 19 the calcium and the phosphorus (inorganic) content of the blood were 9.8 and 4.8 mg. per hundred cubic centimeters, respectively; on June 30 they were 9.1 and 6.3 mg., and on July 1 they were 9.4 and 4.7 mg. On June 19 the chloride content of the plasma (as sodium chloride) was 632 mg., and the cholesterol content of the blood was 167 mg., per hundred cubic centimeters.

From the time he was first examined until 1942, the patient was seen at frequent intervals, and many therapeutic agents were tried. He slowly lost weight and strength and had increasing difficulty in swallowing. Aspiration pneumonia finally resulted in his death in October 1942.

Cases 2 and 3 serve to emphasize the almost monotonous uniformity of the case histories of well developed dystrophia myotonica.

CASE 4 (previously reported by Moleen, Johnson and Dixon[1]).—L. B. L. was first seen at the age of 35 and followed to his death at the age of 56. Two of his brothers were similarly affected. One of these died at the age of 60; no autopsy was done, and the cause of death was stated in the hospital record as myocarditis and auricular fibrillation. The case of the other affected brother is presented as case 5. Another brother was well in 1939, at the age of 63. A somewhat younger brother was well when last heard of, several years ago. One brother died at 19 years of age, of "lead poisoning." A sister died at 64 years of age, of "inward

goiter." Their father died at about 55 years, of "pneumonia," and their mother died at 74 years, of "cancer of the uterus" and "old age."

When seen at the age of 35, the patient stated that at about the age of 27 he noticed a loss of power in the hands and arms, which became progressively weaker and thinner. After about four years, weakness of the legs became evident and likewise progressed steadily. No numbness, tingling or pain was noticed at any time. An occasional twitching of the muscles was noticed. There was no subjective difficulty of vision. Examination at that time revealed an anemic, slender white man with no evident defect of speech. The volume of the tongue was reduced. Symmetric wasting was present throughout the body, but was more evident in the hands, arms and shoulders. The thenar and hypothenar eminences and all the intrinsic mucles of the hands were distinctly wasted. Muscular power in the grasp was greatly reduced. The flexor and extensor power in the arms was markedly diminished. No spontaneous fibrillation was noted, but tapping the pectoral muscle caused a rhythmic wavelike contraction from insertion to origin. In walking, the patient raised the feet higher than was normal in order to clear the toe; the foot was carried forward and struck the floor flat. No ataxia was evident in the movements of the arm or the trunk. Tendon reflexes were generally diminished, but present, and no abnormal reflexes were seen. All forms of sense perception were normal. Visual acuity was normal.

When the patient was examined four years later, at the age of 39, a progression of the condition was observed. A slight difficulty in articulate speech was recognized, especially in cold weather, and the patient stated that the muscles of the tongue would not move as well as before. On an attempt to drink while in a horizontal position, the water ran out of the nose, and if he drank hurriedly, he invariably strangled. The movements of the head were notably weakened, and it had often been necessary for him to assist with his hand when raising his head from the pillow. The depressor muscles of the jaw were weak, and the temporal, pterygoid and masseter muscles were markedly weakened. Wasting of the temporal muscles was definite. The grasp was weaker than when the patient was first seen. The intrinsic muscles of the hands showed more wasting than before, and the eminences were less prominent. The patient found difficulty in relaxing the grasp, the extensor muscles "being too weak to overcome the contraction of the flexors." Flexion of the leg was performed with greater power than extension. Dorsiflexion of the feet was accomplished only with difficulty, and when dependent the feet hung in a position of plantar flexion, or foot drop. Tapping the quadriceps or the sartorius muscle caused a "myotatic" response. The reflexes were more feeble than on the first examination. Tapping the right forearm extensor tendon caused a slight extension of the fingers, which slowly relaxed. Vision had decreased to 15/20 on the right and 15/30 on the left. All forms of sensory perception were normal.

The course of the disease was one of gradual progression, and death occurred at the age of 56 of what was believed to be complete exhaustion.

CASE 5.—In the distribution of the weakness and atrophy and the course of the disease this patient, G. L., was very similar to his brother (case 4). He dated the onset of his weakness from an injury sustained by him at 21 or 22 years of age, when he fell backward over a snow plow and struck his back. The weakness was first noticed in his hands and wrists.

At the age of 40 the patient presented moderate atrophy. The strength of the extensor muscles was definitely weaker than that of the flexor muscles. The tongue was somewhat shriveled in appearance. The reflexes were diminished in degree. Special senses were unimpaired.

The weakness and atrophy of the muscles progressed, and when he was admitted to the hospital at the age of 49 years for bronchopneumonia, the atrophy of the muscles of both upper and lower extremities was fairly marked. At the age of 56 years he was markedly emaciated, and most of the muscles of the body, especially those of the forearms, legs and back, were markedly atrophied. At no time was there any pain or sensory disturbance. He died at the age of 59 of bronchopneumonia.

The conclusion that the last 2 patients had dystrophia myotonica is based on the following evidence: 1. The type of heredity is that found in patients with dystrophia myotonica and differs from that found in patients with other muscular atrophies that occur in adults. Three of the six siblings who lived long enough to manifest the disease were affected. This 50 per cent incidence is commonly seen in families with dystrophia myotonica and is one reason why we believe the disease to be transmitted as a dominant character. The cause of the usual failure to recognize the disease in the parents has been referred to. 2. The distribution of the atrophy was characteristic of dystrophia myotonica. In both patients the atrophy was first noticed in the forearms and hands. Foot drop and inability to bring the head up when lying down were also noted early. The muscles to which these signs are referable will be recognized as the ones which characteristically show early involvement in dystrophia myotonica. The generalized atrophy which occurs as the disease progresses is also characteristic. 3. Voluntary myotonia was present. It is stated that patient L. B. L. (case 4) found difficulty in relaxing his grasp of objects. This difficulty cannot occur if the flexor muscles relax normally, even if the extensor muscles are weak. It occurs only when the contraction of the flexor muscles persists for an abnormally long time after cessation of voluntary effort at contraction—that is, when voluntary myotonia is present. 4. Mechanical myotonia was present. On one examination it was stated that tapping the forearm extensor tendon caused a slight extension of the fingers which slowly relaxed. This is a clear description of a form of mechanical myotonia which can be duplicated in almost any patient with dystrophia myotonica. 5. Some of the dystrophic and endocrine changes which ordinarily complete the picture of dystrophia myotonica were present. These include baldness in both patients and testicular atrophy in L. B. L. (case 4).

AUTOPSY REPORTS AND HISTOLOGIC STUDIES

Case 1 (F. B., aged 44).—This is an abstract of the autopsy record (significant observations only are mentioned).

Gross Findings.—The body was that of a white man; the weight was 168 pounds (76 Kg.) and the length 5 feet 11 inches (180 cm.). The state of nutrition was observed to be fairly good. The atrophy of the sternocleidomastoid muscles and of the muscles of the forearms and hands was found to be well marked. Rigor and livor mortis were slight nine and one-half hours after death. The right pleural cavity contained 200 cc. of clear greenish fluid; the left was empty. Both were free from adhesions. The thyroid gland was small and flattened. The four

parathyroid glands were normally situated but were small, each being 0.3 cm. in the greatest diameter. Several small cysts (0.2 to 0.5 cm. in diameter) were located in the loose connective tissue surrounding the parathyroid glands. The heart weighed 420 Gm. and exhibited no gross abnormalities. Both lungs were large and heavy. The bronchi contained mucopurulent secretion. The surfaces of cut sections of both lungs were very moist and showed purplish red patches on a pink background. The lower lobe of the right lung contained two peripherally located pyramidal grayish solid zones, each about 2 cm. in diameter, The spleen weighed 450 Gm. and was about twice normal size. The splenic substance was dark red, soft and bulging, with numerous white dots scattered throughout. The gastrointestinal tract was normal except for scattered petechial hemorrhages in the pyloric and duodenal mucosa. Both adrenal glands were small, with thin cortical layers. The right weighed 4 Gm. and the left 5 Gm. The testicles were small and soft. Each measured 4.5 by 1.3 by 1.3 cm. Cut surfaces were not grossly remarkable. The skeletal muscles varied from those that were nearly normal in appearance, such as the deltoid, pectoral, abdominal and thigh muscles, to those that were partially or almost completely atrophic. In the latter group were the temporal, the sternocleidomastoid and the arm, forearm and leg extensor groups. These were of a peculiar grayish white color streaked with yellow and were more or less reduced in volume. The temporal and sternocleidomastoid muscles were scarcely recognizable as muscles, being reduced to flat fibrous strands. The peripheral nerves, the brain and the spinal cord were grossly normal. The brain weighed 1,365 Gm. The pituitary gland appeared normal and weighed 0.45 Gm.

Histologic Examination.—The tissues were fixed in Zenker's solution and embedded in paraffin; sections were stained with hematoxylin and eosin unless otherwise stated. Thyroid Gland: The acini were less numerous and more variable in size than those of a normal gland and were lined with low cuboidal epithelium. All were filled with blush-staining colloid. The stroma was fibrous and appeared slightly increased in amount. The appearance was similar to that of senile atrophy.

Parathyroid Glands: In all the glands the stroma showed partial adipose tissue replacement. The parenchyma was composed of "chief" cells, which in some areas formed small acini filled with dull bluish hyaline material. Oxyphilic cells were fairly numerous but lacked regular arrangement. Small cysts lying in the surrounding adipose tissue had a thin fibrous wall without distinct epithelium and a hyaline eosinophilic content.

Heart: Sections of right and left ventricular myocardium showed moderate variability in size of fibers with variation in nuclear size and shape. Cross striations were well defined in all fibers. The number of nuclei was not increased over the normal, and clumping of nuclei as seen in skeletal muscle was lacking.

Lungs: The bronchi were filled with mucus and leukocytes. There was patchy consolidation with acute inflammatory exudate in the alveoli. The peripheral zones of discoloration seen grossly in the lower lobe of the right lung were infarcts, one of which was undergoing marginal organization. There was an unorganized thrombus in the right main pulmonary artery.

Spleen: The sinusoids contained erythrocytes, while the pulp spaces were crowded with erythrocytes and leukocytes of various types, with many polymorphonuclear neutrophils and eosinophils. The malpighian corpuscles were numerous and large.

Gastrointestinal Tract: In sections of the upper end of the esophagus the muscle fibers showed abnormalities characteristic of the disease (fig. 1 *A*). Cross sections of the fibers were circular or oval and varied from 20 to 140 microns in diameter.

The nuclei were apparently increased in number and irregular in shape, and many lay in the substance of the fiber, occasionally in clumps. Many of the larger fibers had a light brownish staining reaction. In these the cut ends of the myofibrils were separated by hyaline substance, and myofibril grouping into Cohnheim's fields was not visible. Cross striations were fully evident except in the large hyalinized fibers, where they sometimes were and sometimes were not visible. When present in these fibers the cross striations were less distinct, more delicate and more closely spaced than those in the other fibers. The sarcolemma might either closely invest the fiber or be completely separated from it. The perimysium was relatively increased in prominence. There was no evidence of inflammation or fibrosis.

Skeletal Muscles: The individual muscle specimens were placed in separate containers at autopsy in order to insure their correct identification. All varied in

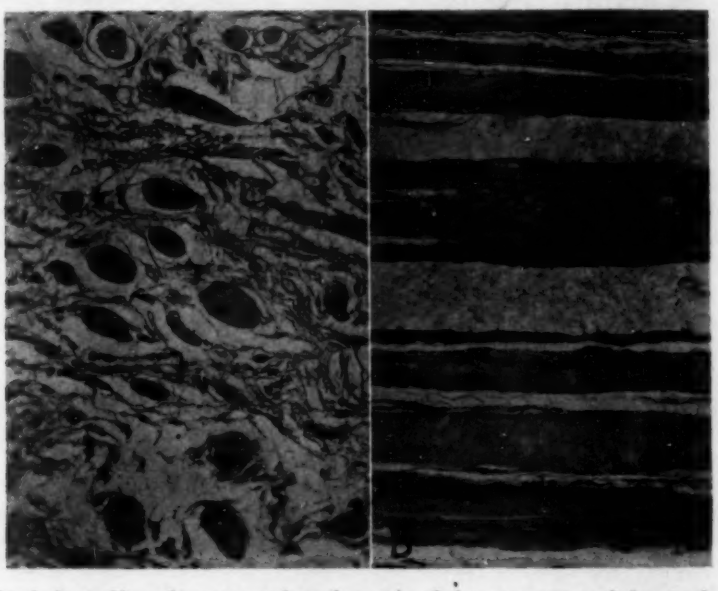

Fig. 1 (case 1).—*A*, cross section of muscle of the upper part of the esophagus; × 125. *B*, flexor muscle of the forearm; × 125.

some degree from the normal, with the exception of the diaphragm, in which no definite changes of muscle fibers were seen. There was, however, a chronic inflammatory reaction along the pleural border. Increasingly severe involvement was observed in the following muscles or muscle groups in the order named: deltoid; quadriceps femoris; pectoralis major; rectus abdominis; arm flexor; leg flexor, extensor and peroneal; arm extensor; temporals and sternocleidomastoid.

In the first four muscles much of the tissue appeared histologically normal, with only an occasional fiber or small group of them being visibly altered. In these the most striking feature was an increase in the number and an alteration in the position of the nuclei. The involved fiber might be increased, normal or diminished in size. The nuclei lay in the sarcoplasm away from the sarcolemma and frequently were arranged in rows along the long axis of the fiber. Cross striations were visible and might be coarse but were often delicate and closely

spaced (fig. 1 *B*). The lesion might be noted at separate points along a single fiber, with normal-appearing intervening structure. Occasionally a coarsely vacuolated fiber was seen.

In the other muscles studied, the amount of tissue involved was greater, and the lesions were more severe. As the process became more marked, the fibers became rounded as seen in cross section. While some were normal or increased in diameter, the majority were smaller, ranging from 20 to 120 microns in diameter. The nuclei showed variation in intensity of staining, and in the advanced lesions many were hyperchromatic. Cohnheim's fields disappeared in many fibers about the periphery, persisting in the central zone. In other instances the cross section appeared hyaline or vacuolated, no myofibrils being visible. The substance of such fibers stained a deeper, faintly bluish pink. An extreme degree of involvement was seen in the temporal muscle. Here the fibers were small, although giant fibers were present, with long rows of hyperchromatic nuclei, which were often clumped. Most of these retained both cross striation and visible myofibrils. Segments of involved fibers might be thinned out to filament dimensions yet retain cross striation throughout. Fatty metamorphosis was not noted in any of the skeletal muscles. The blood vessels appeared normal except for certain small veins in the muscles of the lower extremities in which recent thrombosis had taken place. By the use of Bielschowsky's and of Bodian's staining methods, the small myelinated nerve trunks within the muscles were seen to be somewhat lacking in number of both myelinated and nonmyelinated fibers as compared with the normal. Nonmyelinated nerve fibrils ramified throughout both the affected and the nonaffected muscles. No relationship between distribution of nerve fibrils and normal or atrophic muscle fibers could be established.

Spleen: The organ appeared congested. The red pulp contained numerous leukocytes, many of which were eosinophilic polymorphonuclears.

Liver: No significant changes were present.

Pancreas: This organ appeared normal.

Adrenal Glands: Both glands were alike. The cortices were reduced to approximately half the normal thickness. There was reduction in number of cells in the fascicular zone, with lack of lipid storage. Irregularly spaced groups of fascicular cells had coarsely vacuolated cytoplasm. Those nearest the medullary border contained brown granular pigment. Single cells and groups of them appeared hypertrophied; others (the remainder), more or less atrophic. The glomerular layer was uneven; it appeared atrophic, with zones of cellular hypertrophy. The cells were hyperchromatic and more deeply basophilic than the normal (compared with those of a man of the same age killed in an automobile accident). The zona reticularis was poorly defined and variable in thickness. In places it underlay the glomerular zone directly, owing to the apparent disappearance of the fascicular layer. Cells contained brownish granular pigment, and occasional cells were vacuolated. "Dark" cells predominated. Pyknosis of nuclei with shrinkage of cells was evident along the medullary border (fig. 2 *A*).

Kidneys: These were essentially normal.

Urinary Bladder: This was normal.

Prostate: This was not remarkable.

Testicles: The right testicle contained a few atrophic tubules, but for the most part the tubules were normal and contained spermatozoa. The left showed interstitial edema, with only a few atrophic tubules among the normal ones. There was an apparent increase in number of interstitial cells (fig. 2 *B*). The right epididymis was normal. The left contained calcified debris within the ducts.

Pituitary Gland: The fibers of the posterior lobe were less compact than usual, with granular material present in the interstices. The pars intermedia contained

several "colloid"-filled cysts. There was an apparent reduction of the total number of cells in the anterior lobe. The cells formed acini, which in many instances were filled with solid plugs of basophilic hyaline substance. Basophilic cells predominated with only an occasional eosinophilic cell present (fig. 3 *A*).

Cervical Ganglions: These appeared normal.

Bone (vertebra and rib): These specimens appeared normal.

Pineal Gland: This was anatomically normal, with the usual number of calcospherites lodged within it.

Central Nervous System: There were no significant alterations in the brain. The ganglion cells of the anterior horn appeared to be reduced in number.

Nerve Trunks: The medium-sized trunks (posterior tibial, for instance) appeared to be somewhat deficient in number of nerve fibers.

Sympathetic Ganglions: These appeared normal.

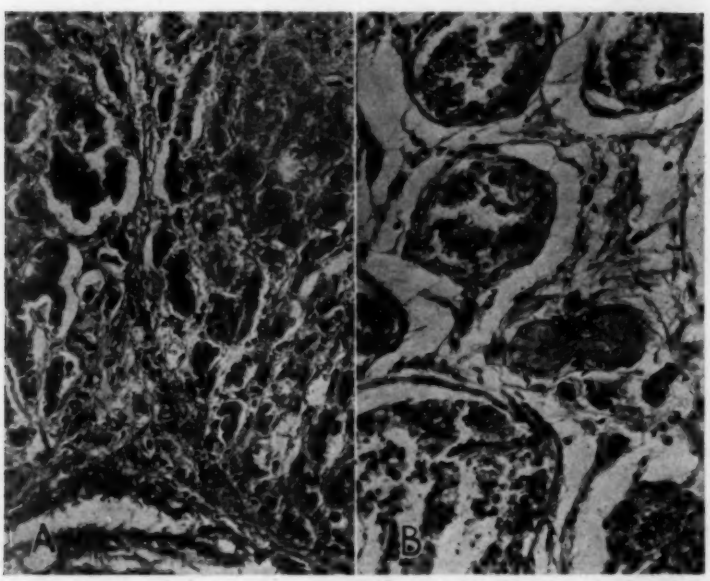

Fig. 2 (case 1).—*A*, adrenal cortex; × 125. *B*, testis; × 125.

Case 2 (O. M., aged 44).—This autopsy was performed at the Denver General Hospital. The body is stated to have been well developed and well nourished. There is no statement regarding muscle wasting and no description of the external genitalia. The heart weighed 340 Gm. and appeared normal grossly. The adrenal glands were normal except for a cortical adenoma, 1 cm. in diameter, present in the left gland.

The brain weighed 1,350 Gm. There was extensive subdural hemorrhage, accompanying skull fracture. The pituitary gland, the thyroid gland and the spinal cord are not described grossly. Microscopically, the brain showed no changes except those produced by recent trauma. The pituitary gland appeared normal. The adrenal gland was normal except for the presence of the cortical adenoma.

There were two sections of skeletal muscle, one of which appeared entirely normal. The other showed fibers varying in size from a few larger than normal in diameter to many which were smaller than normal. Tapering, wavy and segmentally constricted fibers were seen. There was an increase in the number of

nuclei, and some were arranged in rows apparently within the sarcoplasm. Cross striations were present except in a few of the wavy fibers in which the sarcoplasm was hyalinized. Clumping of nuclei was not observed. The muscle changes were of the same type as in the other cases.

CASE 3 (J. B., aged 63).—The body was emaciated, with very marked wasting of the muscles of the face, the neck, the shoulder girdle and the extremities. Atrophy of the tongue, the extrinsic muscles of the larynx and the skeletal muscle of the upper portion of the esophagus was present. The heart was essentially normal. There was a moderate degree of atherosclerosis of the coronary arteries and the aorta. Both lungs were heavy and wet; the cut surfaces were a mottled purplish red and exuded much watery pink fluid. The bronchi were filled with thick mucopurulent secretion. The adrenal glands together, with some attached adipose tissue, weighed 9 Gm. The cut surfaces showed thin, uneven, poorly

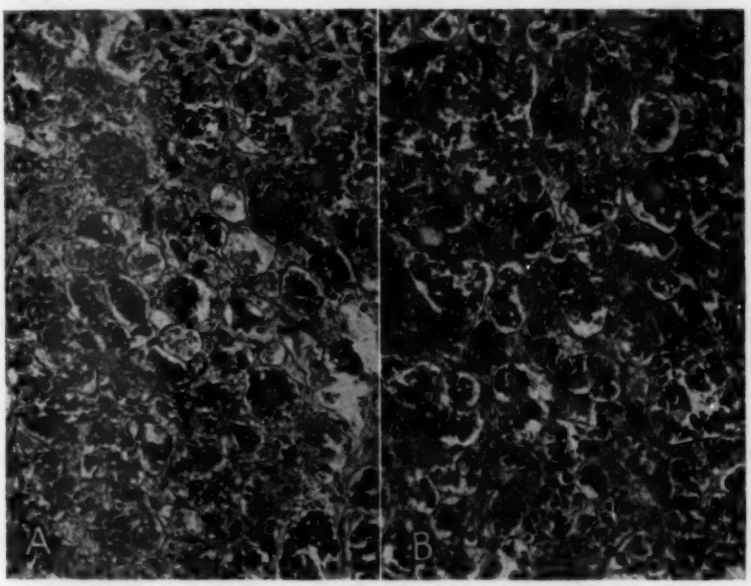

Fig. 3.—*A*, anterior lobe of the hypophysis in case 1; × 125. *B*, anterior lobe of the hypophysis in case 3; × 125.

demarcated yellow cortices. The prostate was small and soft. Both testicles were small and very soft; each weighed 30 Gm. The brain and the spinal cord were grossly normal. The pituitary gland weighed 0.5 Gm.

Microscopically, changes in the skeletal muscles were identical with those found in the other cases. The thyroid and parathyroid glands were partially atrophic. The thymus showed complete involution. The lungs showed confluent bronchopneumonia with foreign material (cellulose fibers) in the bronchi and alveoli, indicating that aspiration of food particles had occurred. The adrenal glands showed cortical atrophy with patchy hyperplasia of the zona glomerulosa and the zona fascicularis and irregular lipid storage. The testes were completely atrophic and did not show the apparent increase in interstitial cells observed in the other cases. The prostate was entirely lacking in glandular hyperplasia. The anterior lobe of the pituitary gland was sharply congested, with fewer than the usual number of cells, but all cell types were present and appeared in approximately

normal distribution (fig. 3 *B*). Hyalin-filled or "colloid"-filled acini were numerous. The posterior lobe appeared normal.

Case 4 (L. B. L., aged 56; case reported by Moleen, Johnson and Dixon [1]).—The body showed extreme emaciation with marked muscle wasting, but the heart weighed 310 Gm. and was normal in size and shape. The right adrenal gland weighed 8 Gm.; the left 8.5 Gm. The cortices were paler and thinner than they are normally. The testicles were small and soft. The lumbar enlargement of the spinal cord was soft and shrunken. The pituitary gland was small; the weight was not recorded. A gross description of the thyroid gland is not given; microscopically the gland was found to contain several large colloid-filled cysts. The remainder of the gland was made up of large acini solidly filled with colloid, lined by low cuboidal epithelium and apparently inactive.

The changes in the skeletal muscles differed in no way from those described in case 1. The cardiac muscle did not exhibit any such changes. The adrenal glands both showed decreased cortical cellularity. The number of cells in the zona glomerulosa and the zona fasciculata was reduced, with lipid-bearing cells arranged in groups. The zona reticularis was broad, but not compact, with many cells containing light brown pigment. The medulla of each appeared normal. The spinal cord and peripheral nerve changes have been described in detail by Dixon,[1] who expressed the belief that they were the primary pathologic lesion. ("Non-inflammatory degeneration of the myelinated elements of the peripheral nerves appears to constitute the primary pathologic lesion.") Guillain, Bertrand and Rouques [8] expressed the belief that the nerve changes are secondary.

The pituitary gland was not entirely normal. There was partial fibrosis, with reduction in number of cells of the anterior lobe and relatively greater reduction in number of the acidophillic cells.

The testicles were practically completely atrophic.

Case 5 (G. L., aged 59).—There was marked emaciation with great wasting of muscles. The testicles were about one-half normal size. The heart weighed 250 Gm. and was essentially normal in gross appearance. Both lungs were heavy, with patchy consolidation throughout. The adrenal glands were normal grossly (weight not recorded). The thyroid gland, the pituitary gland, the brain, the spinal cord and the testicles are not described.

The skeletal muscle changes were similar to those observed in the other cases. The heart muscle showed an increased amount of lipochrome pigment but was otherwise normal. The lungs showed confluent bronchopneumonia. In the adrenal glands there was a reduction in thickness of the zona fasciculata, with well defined island grouping of cells containing lipoid material, but the degree of alteration was not so great as in some of the other cases. The testicles showed almost complete atrophy, only a few seminiferous tubules remaining, with no evidence of spermatogenesis.

Sections of several nerve trunks all showed reduction in number of myelinated fibers.

There were no sections of brain, spinal cord or pituitary gland.

COMMENT AND SUMMARY

Five cases of dystrophia myotonica were studied pathologically so far as tissues were available. In one instance the central nervous system was not examined, and in another not all of the organs were

8. Guillain, G.; Bertrand, I., and Rouques, L.: Ann. de méd. **31**:180, 1932.

saved for microscopic study. Consequently the cases cannot be prepresented on a fully comparative basis. However, we believe that enough data have been obtained to justify a report.

In addition to the lesion of the skeletal muscles a number of investigators have described changes occurring in the endocrine glands and the central and peripheral nervous systems. These changes occur but are inconstant and do not permit interpretation at the present time.

In 4 of the 5 cases the pituitary gland was available for study. In 2 instances the anterior lobe was somewhat atrophic, with a relative increase in number of basophilic cells. In the other 2 the gland was apparently within normal limits.

In 4 of the 5 cases the adrenal glands showed a degree of cortical atrophy, particularly affecting the zone fasciculata, with island grouping of cells containing lipoid material. In the fifth case the adrenal glands were normal except for a cortical adenoma of the left gland.

In 4 cases the thyroid gland appeared to be less than normally active. In the remaining case it was not available for study.

The brain and the spinal cord were studied in 3 cases (1, 3 and 4). The spinal cords appeared deficient in number of anterior horn cells, with segmental differences in number of these cells. Peripheral nerve trunks were studied in 3 cases, and were found to be deficient in number of myelinated nerve fibers.

An effort was made in 1 case to demonstrate the nonmyelinated nerve fibrils and motor end plates of skeletal muscles. The results were not entirely satisfactory because of technical difficulties. However, nonmyelinated fibrils were observed to ramify equally in affected and nonaffected muscles, and motor end plates as well as sensory endings were found in muscles in all stages of involvement.

The testicles were almost completely atrophic in 3 cases, slightly atrophic in 1 and were not studied in 1 case.

The muscle lesion is characterized by irregularity of distribution within the perimysium, by apparent increase in number of nuclei, by alteration in spacing and location of nuclei and by striking changes in size of individual fibers. The late changes include fibrosis and adipose tissue replacement and are considered to be secondary.

No explanation of the disease can be advanced from a pathologic standpoint, although it may be stated that there is associated hypoplasia or partial atrophy of the anterior lobe of the pituitary gland, the thyroid gland, the adrenal cortex and the testicles.

The fact that severe bronchopneumonia was present in 3 of the patients who died naturally suggests that bronchopneumonia is a common terminal event, to which the patients predispose themselves when they aspirate food and other material from the pharynx as a result of myotonic changes in the muscles of deglutition.

Case Reports

MIXED SQUAMOUS CELL CARCINOMA AND PAPILLARY ADENOCARCINOMA (ADENOACANTHOMA) OF THE THYROID GLAND

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CANCER of the thyroid gland is infrequent, occurring in 1 to 2 per cent of thyroid glands removed surgically. In a series of 3,389 thyroidectomies at the Lahey Clinic, 1916 to 1937, Clute and Smith [1] found 67 cancers, an incidence of 1.68 per cent. Squamous cell carcinoma of the thyroid gland is rare. In 774 cases of cancer of the thyroid gland reported from the Mayo Clinic in the period from 1907 to 1937 by Pemberton [2] the frequency was: papillary adenocarcinoma, 30 per cent; adenocarcinoma in adenoma (cancerous adenoma), 38.1 per cent; diffuse adenocarcinoma, 30.4 per cent; sarcoma, 0.75 per cent, and squamous cell carcinoma, 0.75 per cent. Davis [3] reported 2 cases of squamous cell epithelioma in a series of 50 cases of cancer of the thyroid gland. Other reports of cases of carcinoma of the thyroid gland include single cases of squamous cell carcinoma.[4]

The simultaneous occurrence of squamous cell carcinoma and any other type of carcinoma of the thyroid gland has not been reported. A case of mixed squamous cell carcinoma and papillary adenocarcinoma is therefore considered of sufficient interest to report.

REPORT OF A CASE

This 61 year old Greek man was admitted to the Royal Victoria Hospital, Montreal, April 7, 1946, complaining of anginal attacks and dyspnea of three years' duration, hoarseness of three months' and hemoptysis of two months' duration. He had been hospitalized on two previous occasions for "heart trouble" and gallstones.

In January 1946 his throat became sore and hoarseness developed. Shortly after, hemoptysis appeared, and paralysis of the right vocal cord was discovered. In March fixation of the larynx was noted, and the impression was that of a neoplasm of the right lobe of the thyroid gland.

On his admission to the hospital, examination revealed a blood pressure of 138 systolic and 70 diastolic, a palpable liver and spleen and paralysis of the right vocal cord. Bronchoscopy showed a normal trachea and bronchi. A firm nodule was palpable in the right lobe of the thyroid gland, but surgical removal was considered inadvisable. The basal metabolic rate was +1 per cent. He was discharged on April 25 as improved.

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1. Clute, H. M., and Smith, L. W.: Arch. Surg. **18**:1, 1929.
2. Pemberton, J. de J.. Surg., Gynec. & Obst. **69**:417, 1939.
3. Davis, H. A.: Arch. Surg. **39**:435, 1939.
4. Smith, L. W.; Pool, E. H., and Olcott, C. T.: Am. J. Cancer **20**:1, 1934. Clute and Smith.[1]

His final admission was on September 12. At this time he complained of expiratory dyspnea of two weeks' duration, which had increased markedly on September 7.

His breathing was labored, particularly on inspiration, and stridor was present. On the right side of the neck, just above the medial extremity of the clavicle, a firm mass, ¾ inch (about 2 cm.) in diameter, was palpable. The diagnostic impression was that of mechanical obstruction of the larynx or the trachea. A hemogram on September 14 showed red blood cells, 5,400,000; hemoglobin, 106 per cent; white blood cells, 7,400, and sedimentation rate, 27 mm. in one hour (29 corrected as to cell volume.)

On September 17 bronchoscopy was performed. One-half inch below the vocal cords there was a mass projecting into the trachea from the right side, nodular and vascular in appearance, filling the trachea except for a slit 2 mm. in width along the left wall. As soon as the mass was touched, it began to ooze. A biopsy was made, after which the patient stopped breathing and had to be intubated. Tracheotomy was then carried out. The impression was that of carcinoma involving the right lobe of the thyroid gland and secondary infiltration of the trachea.

The same evening the patient complained of a choking sensation; suction was used, but the tracheotomy tube did not appear to be blocked. His face became swollen and cyanotic, and within fifteen minutes he died.

Autopsy (fourteen and one-half hours post mortem; limited to thorax and abdomen).—Only those findings pertinent to the case will be described in detail.

The face was livid and swollen. Interstitial emphysema was present in the neck and the mediastinum. A tracheotomy tube was lying in the interstitial tissue anterior to the trachea. A small amount of blood was present in the trachea.

The right lobe of the thyroid gland was one and a half times the size of the left lobe, firm in consistency and adherent to the surrounding structures. On section most of the peripheral portion was seen to consist of normal tissue, but medially the gland had been replaced by an irregular nodular mass, 3 cm. in diameter. It was firm in consistency and white with small yellow areas. Posteriorly the nodule extended to the capsule of the gland but had not penetrated it. Medially the growth had ulcerated into the trachea just inferior to the cricoid cartilage, and within the trachea there was a reddish, friable mass, 1.5 by 1 by 1 cm. A tracheotomy incision was present, immediately inferior to the mass in the trachea. The right recurrent laryngeal nerve was traced upward and lost in the tumor mass. The left lobe of the thyroid gland was reddish brown and moderately firm in consistency and on section showed normal structure.

The heart showed hypertrophy of the left ventricle (460 Gm.) and fibrosis of the interventricular septum. Moderate coronary arteriosclerosis was present, involving especially the anterior descending branch, but no occlusion was demonstrated. There was chronic passive congestion of the lungs, the liver, the spleen and the kidneys. No metastases of tumor were found.

Incidental findings included cholelithiasis, obsolete tuberculosis of bronchial lymph nodes and of the spleen, and hyperplasia of the prostate.

Microscopic Observations.—The right lobe of the thyroid gland containcd a small amount of relatively normal tissue immediately beneath the capsule. The acini varied considerably in size and were well filled with eosinophilic colloid. There was a slight increase of interstitial fibrous tissue. In a region more remote from the capsule, the acini were poorly filled with pale colloid, and there was a moderate increase in hyaline connective tissue with calcification, small areas of hemorrhage and focal collections of lymphocytes and plasma cells.

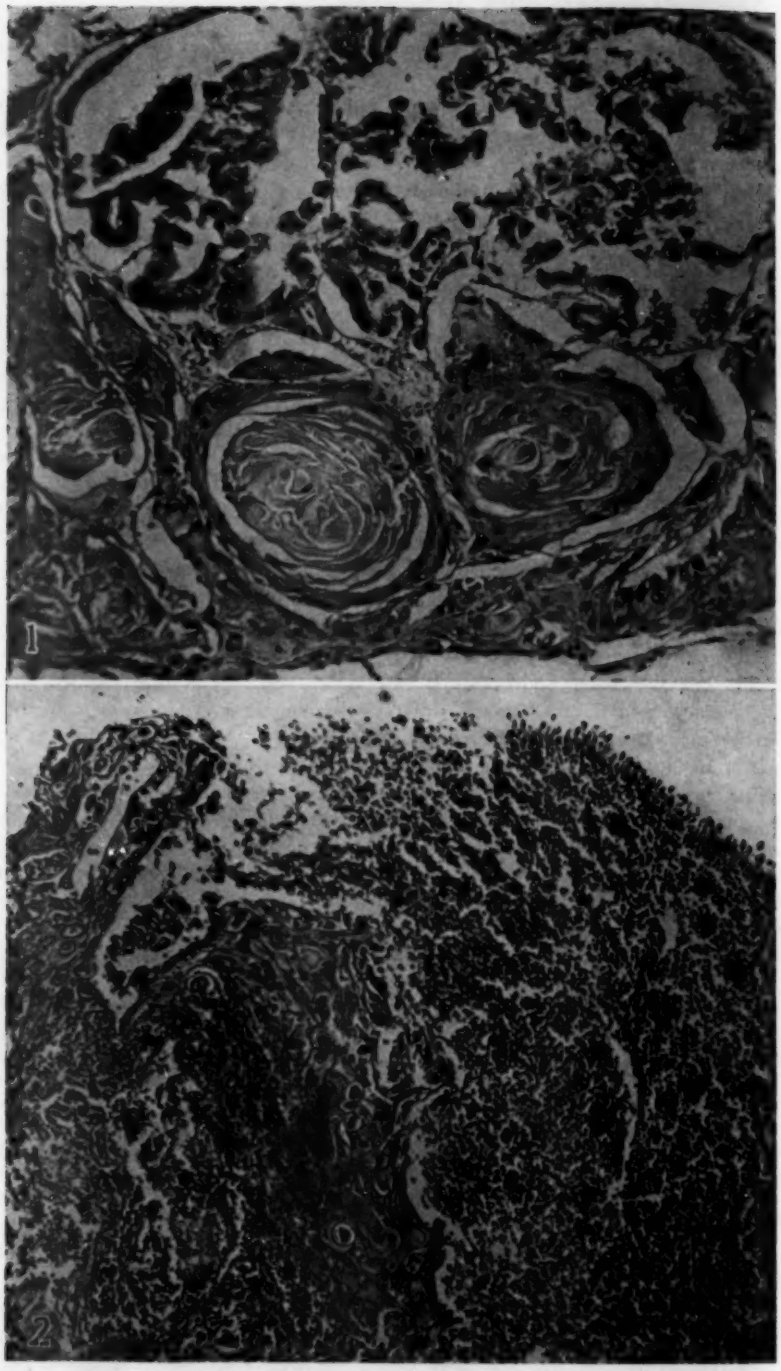

Fig. 1.—Photomicrograph of tumor in the right lobe of the thyroid gland showing squamous cell carcinoma with pearl formation in the lower half of the field and papillary adenocarcinoma in the upper half. Central necrosis is evident in the squamous cell carcinoma. Hematoxylin-eosin. × 110.

Fig. 2.—Photomicrograph of tumor in the area of tracheal ulceration showing tracheal epithelium, in the upper right of the field, which is interrupted by squamous cell carcinoma, with no evidence of transition. Hematoxylin-eosin. × 112.

Blending with this altered thyroid tissue were two distinct types of neoplastic epithelium and intermediate gradations. The predominant type consisted of large, irregular acini with papillary projections, lined by darkly staining high columnar epithelium. The nuclei, which were located near the basement membrane, were elongated, hyperchromatic and vesicular, and showed few mitotic figures. The second type, comprising 40 to 50 per cent of the tumor, consisted of islands of keratinizing squamous epithelium with pearl formation and areas of central necrosis. The nuclei were large and vesicular, with distinct nucleoli. The cytoplasm was pale staining and vacuolated, and intercellular bridges were present. In other areas there were large pleomorphic cells with one or more very large vesicular or hyperchromatic nuclei and faintly basophilic cytoplasm, which trailed off into processes and in some cases contained phagocytosed polymorphonuclear leukocytes. In areas of the tumor lying between adenocarcinoma and squamous cell carcinoma acini were present which showed piling up of epithelium to a varying degree, but it was not possible to demonstrate an acinus that was clearly lined by columnar epithelium in one area and by stratified squamous epithelium in another (fig. 1).

The stroma varied in different portions of the tumor. In the acinous areas it occurred as fibrous septums between groups of acini, and in the squamous areas, as dense collagenous connective tissue surrounding larger or smaller islands of epithelial cells. There was a moderate infiltration of lymphocytes, plasma cells and polymorphonuclear leukocytes, the latter in relation to areas of necrosis.

Section of the area of tracheal ulceration showed the pseudostratified ciliated columnar epithelium of the trachea interrupted by keratinizing squamous cell carcinoma penetrating from without (fig. 2). All the tumor tissue which projected into the trachea was of the squamous cell type, but papillary adenocarcinoma was in close relationship and lay between the cartilage of the tracheal ring and the mucosa. At one point the mucosa overlying the adenocarcinoma was necrotic. There were hyperemia, small areas of hemorrhage and a marked infiltration of polymorphonuclear leukocytes, plasma cells and eosinophilic leukocytes in the submucosa of the trachea and the projecting portion of tumor.

Although the tumor lay in close relationship to capillaries, no invasion of veins or lymphatic channels was observed and sections of the regional lymph nodes contained no tumor.

In the left lobe of the thyroid gland the acini were fairly uniform in size, lined by low cuboidal epithelium and well filled with lamellated eosinophilic colloid. The capsule was normal, and the interstitial tissue and vessels showed no abnormality.

COMMENT

This case was interpreted as one of mixed squamous cell carcinoma and papillary adenocarcinoma of the thyroid gland with local invasion and ulceration of the trachea. The possibility that the squamous cell carcinoma may have originated from respiratory epithelium which had undergone squamous cell metaplasia must, however, be considered.

Carcinoma of the trachea is a rare type of neoplasm, but of 95 cases that have been described [5] 42 were of the squamous cell type. However, by distribution, only 35 per cent of the lesions occurred in the upper third, and of these more than one half were on the posterior wall of the trachea. Thus the site of the tumor in this case is not a common site of tracheal carcinoma.

5. Olsen, A. M.: Arch. Otolaryng. **30**:615, 1939.

However, one of the most significant points against primary squamous cell carcinoma of the trachea is the mode of the neoplastic ulceration of the trachea. The normal pseudostratified ciliated columnar epithelium was suddenly interrupted and inverted into the tracheal lumen by squamous cell carcinoma. There was no suggestion of gradual transition between this epithelium and the squamous cell type (fig. 2), and although the tumor which projected into the tracheal lumen was purely squamous cell in type, well differentiated papillary adenocarcinoma had invaded the tracheal wall, lying between tracheal cartilage and mucosa and at one point producing necrosis of the overlying mucosa. In addition, the main mass of squamous cell carcinoma was in the right lobe of the thyroid gland and only a relatively small portion was within the trachea.

Moreover, five months before death the trachea was normal on bronchoscopic examination, one month after a firm nodule had been discovered in the right lobe of the thyroid gland, which was fixed to the larynx. From these facts alone, spread from thyroid gland to trachea is more plausible than the reverse.

The matter of whether the squamous cell carcinoma had its origin in the thyroid gland is unsettled. Ewing [6] stated that such a lesion arises not from branchial remnants but from the thyroglossal duct and its pyramidal process.

Jaffé [7] expressed the belief that epithelial metaplasia is the source of squamous cell carcinoma of the thyroid gland, and he reported 3 cases of sclerosis of the thyroid gland in which cuboid epithelium of the follicles changed to squamous epithelium. He also reported 1 case of metastatic abscess of the thyroid gland in which islands of squamous epithelium were found, some showing evidence of central keratinization, which were connected with follicles by a single layer of flattened epithelial cells. He considered that there was no indication that the squamous epithelial cells in the thyroid gland may have been derived from embryonic structures, such as the thyroglossal duct, the ultimobranchial body or the branchial clefts. He quoted Lubarsch,[8] who stated that for metaplasia to occur it is necessary that old cells degenerate and that young and not yet fully differentiated cells proliferate and meet with changed environmental conditions. Jaffé continued that if these requirements are fulfilled in the thyroid gland, the follicular epithelium may change its character as epithelial cells do in other organs, and that if the proliferation of metaplastic epithelium assumes the properties of autochthonous growth, there will result neoplasms which suggest a dysontogenetic origin. Three cases of squamous cell carcinoma of the thyroid gland were reported by him, in one of which follicular epithelium transformed to squamous epithelium.

Englisch and Slany [9] reported an unusual epithelial tumor of the thyroid gland, consisting of acini lined by high columnar epithelium, with buds of squamous epithelium originating at the base of the former and protruding into the glandular lumen or into connective tissue.

6. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 990.

7. Jaffé, R. H.: Arch. Path. **23**:821, 1937.

8. Lubarsch, O.: Deutsche, Ztschr. f. Chir. **227**:48, 1930; cited by Jaffé.[7]

9. Englisch, E., and Slany, A.: Zentralbl. f. Chir. **63**:2830, 1936.

They considered this to be an adenocancroid (adenoacanthoma) and not metaplasia.

In the present case, careful examination of numerous sections failed to reveal all stages of transition between columnar epithelium and stratified squamous epithelium within a single acinus. It is difficult, however, to explain the variable tumor tissues on any basis other than a combination of metaplasia and neoplasia of the epithelium of the thyroid gland.

Herxheimer, in reporting a case of carcinoma of the pylorus in which there was intermingling of squamous cell carcinoma and adenocarcinoma, suggested the name "adenoacanthoma." [10] Tumors of this type have been reported occurring in the stomach, the uterus, the breast, the gallbladder, the pancreas, the large bowel and the lung.[11] In the present case, although the two main types of neoplastic epithelium occurred in zones, with close intermingling only in the areas of apposition, perhaps the tumor is best classified as an adenoacanthoma of the thyroid gland.

SUMMARY

A case of mixed squamous cell carcinoma and papillary adenocarcinoma of the thyroid gland, with a portion extending into the trachea, is presented. The possibility that the squamous cell carcinoma may have originated in the trachea and invaded the thyroid gland is considered, but it is concluded that both types of carcinoma arose in the thyroid gland.

Whether the squamous cell carcinoma of the thyroid gland arises from embryonic remnants or as a result of metaplasia is unsettled. The present case is interpreted as an adenoacanthoma of the thyroid gland.

10. Pasternack, J. G.: Am. J. Path. **11**:541, 1935.

11. Boyd, W.: Surgical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1942, p. 140.

PRIMARY NEOPLASMS OF THE HEART

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ALL TUMORS of the heart are rare, and primary tumors are even more so. Perlstein,[1] Goldstein,[2] Yater[3] and Mahaim[4] have reviewed the subject extensively, and the latter, in 1945, classified and reported on 413 cases of primary myocardial and pericardial tumors which he found during an exacting study of all the available literature. Since the publication of Mahaim's report, at least 9 more cases of primary tumor of the heart have been recorded in the literature (Straus and Merliss[5]; Batchelor and Maun[6]; Anderson and Dymtryk[7]; Pratt-Thomas[8]; Woll and Vickery[9]), and these together with the 2 cases reported here bring the total to 424.

The incidence of primary tumors as determined from autopsy material has been a matter of considerable variation. Ravid and Sachs[10] found the incidence to be 0.05 per cent during a survey of their own autopsy material. However, variations from this figure have been wide, and this was explained by Straus and Merliss[5] as being due to an insufficient number of reported cases. Mahaim[4] stated that a more authentic picture of the frequency of such tumors would be obtained if the figures were based not on the general autopsy figures but on those of patients proved to have heart disease. At the Winnipeg General Hospital during the past ten years 648 nonviable fetuses have been examined, totaling 1.4 per cent of all surgical specimens examined, and no cardiac neoplasms have been encountered apart from the one reported here. Since 1916, in the same hospital, 6,275 autopsies have been conducted, including 509 made on viable fetuses and newborn infants, and only 1 primary tumor of the heart was recorded

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From the Department of Pathology, Faculty of Medicine, University of Manitoba.

1. Perlstein, L.: Am. J. M. Sc. **156**:214, 1918.

2. Goldstein, H. I.: New York M. J. **115**:97, 1922.

3. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.

4. Mahaim, I.: Les tumeurs et les polpes du coeur: Etude anatomoclinique, Paris, Masson & Cie, 1945.

5. Straus, R., and Merliss, R.: Arch. Path. **39**:74, 1945.

6. Batchelor, T. M., and Maun, M. E.: Arch. Path. **39**:67, 1945.

7. Anderson, W. A. D., and Dymtryk, E. T.: Am. J. Path. **22**:337, 1946.

8. Pratt-Thomas, H. R.: Am. J. Path. **23**:189, 1947.

9. Woll, E., and Vickery, A. L.: Arch. Path. **43**:244, 1947.

10. Ravid, J. M., and Sachs, J.: Am. Heart J. **26**:385, 1943.

(case 1). With regard to malignancy, Yater [3] reported 20 per cent of his series to be cancers; the remainder were not cancerous. Larson and Sheppard [11] in 1938 reported 29 per cent to be cancers.

Primary sarcoma of the heart was reviewed by Perlstein [1] in 1918, and of all the reported cases up until that time, he considered only 30 to have been cases of true sarcoma; he added 1 case of his own. Mahaim [4] in 1945 reviewed 87 cases, and Woll and Vickery [9] have added the eighty-eighth to the list. Since primary tumors of the heart are mesoblastic in origin, the following types of sarcoma have been described: fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, pleomorphic cell sarcoma, lymphangioendothelioma and hemangioendothelioma. The last two types are of extreme rarity. The primary tumor is found most often involving the auricles of the heart, with a greater frequency of involvement of the right side.

Of the noncancerous tumors, the myxoma is probably the most common. It is a pale, soft, pedunculated tumor, most often found in the left auricle. In the past considerable discussion has arisen with regard to this tumor. Some have considered it to be a degenerating form of an organizing thrombus in which imbibition has occurred. However, Ewing [12] and many others have held that this is not the case, finding it difficult to believe that an organizing thrombus could assume the appearance of a true myxoma. A second noncancerous tumor is the fibroma, of which 37 cases have been reported. It is a small, firm tumor arising in the subendothelial tissues; it is often attached to valves and may be polypoid in type. Occasionally the characteristics of fibroma are combined with those of myxoma in the same tumor. Then the name "fibromyxoma" has been applied. Lipoma of the heart is rare, reports of only 14 cases having been found in the literature by Mahaim.

A fourth type of noncancerous tumor is the rhabdomyoma or congenital nodular glycogenic tumor as suggested by Batchelor and Maun [6] in their excellent review of tumors of this type. Yater,[3] in his review, described three main subtypes: (*a*) solitary, located near the apex of the heart, (*b*) multiple, in which moderately sized discrete nodules are scattered throughout the myocardium, and (*c*) diffuse, consisting of numerous small nodules scattered indiscriminately throughout the myocardium. The multiple and the diffuse form are frequently associated with diffuse sclerosis of the cerebral cortex, multiple neuroglial rests in the spinal meninges and embryonic nodules of the kidney. Microscopically, the cells are of an embryonal type; they are tubular in shape and attain large size. Within the cells are large glycogen-containing vacuoles and a nucleus suspended in a weblike arrangement of cytoplasmic strands. Both in these and in the cell wall may be seen the characteristic striations of striated muscle. The majority believe that these cells are embryonal in type but may reach a high degree of maturity.

11. Larson, C. P., and Sheppard, J. A.: Arch. Path. **26**:717, 1938.

12. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 187.

REPORT OF CASES

CASE 1.—*Rhabdomyosarcoma of the Heart.*—The tumor occurred in a boy aged 14 years. He was first seen on May 6, 1936 because of cough, which was more severe on lying down, slight whitish sputum and increasing fatigue of three weeks' duration. There was no history of tuberculosis. Physical examination revealed only that a few small lymph nodes were palpable in the back of the neck. Roentgenograms of the chest disclosed a bulging mass in the superior mediastinum. The blood cell count showed 8,400 white blood cells, with eosinophils amounting to 9.5 per cent; the latter subsequently fell to 4 per cent. A tentative diagnosis of Hodgkin's sarcoma was made. Shortly after admission he contracted acute tonsillitis, which improved under appropriate treatment. On June 8 roentgen therapy was instituted and continued for about three weeks. At the end of the course of treatment there was no noticeable change in the mediastinal mass, but as the patient was feeling well he was discharged from the hospital without further treatment.

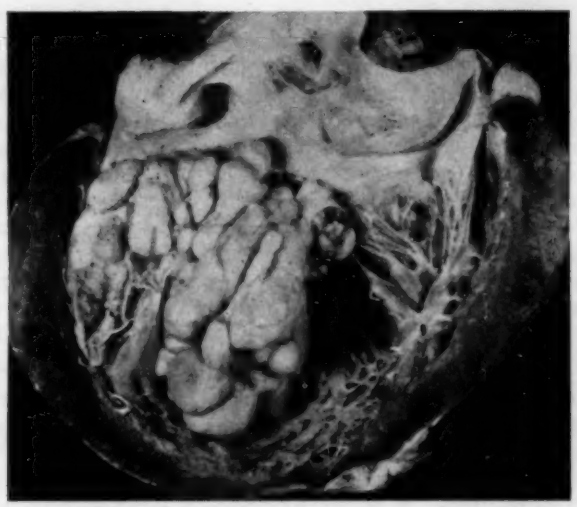

Fig. 1 (case 1).—Primary rhabdomyosarcoma of the heart involving the anterior wall of the left ventricle.

He was readmitted on August 14 with a diagnosis of bronchopneumonia. Roentgen examination of his chest showed that there had been essentially no change in the mediastinal mass since the last examination, and in consideration of this the diagnosis was changed from Hodgkin's sarcoma to benign lipoma or a cyst of the mediastinum of undetermined type. The patient was discharged on September 21.

He was not seen again until he was admitted to the hospital on July 8, 1938 in a moribund condition. He died on July 9 before further examination could be made.

Autopsy.—The body was that of a thin white boy 14 years old. There were no palpable lymph nodes. There was no edema. The veins of the chest were prominent.

When the thoracic cavity was opened, a huge mass was seen filling the mediastinum, completely replacing the thymus and extending down to a short

distance above the diaphragm. From side to side it measured 22 cm. It compressed both lungs, pushing them down. On section the mass was composed of several lobulated portions, and the cut surface was pale pinkish, moist, with large areas replaced by mucin, which was yellow in color. The trachea was pushed to the right, and the esophagus was compressed by the mass.

The pericardial cavity was pushed down. It contained a normal quantity of serous fluid.

The heart weighed 350 Gm. A slight bulging could be seen about the middle of the anterior wall of the left ventricle. In the apex the bulge was slightly paler than the rest of the heart. The right side of the heart was normal and contained only postmortem clots. The left auricle was normal. A large yellowish polypoid tumor filled the cavity of the left ventricle, growing from the anterior wall of the myocardium. It measured 5.5 cm. in diameter. The base of the tumor involved the anterior wall and the interventricular septum of the left ventricle and extended to the anterior cusp of the mitral valve but did not involve it. The remaining findings were irrelevant.

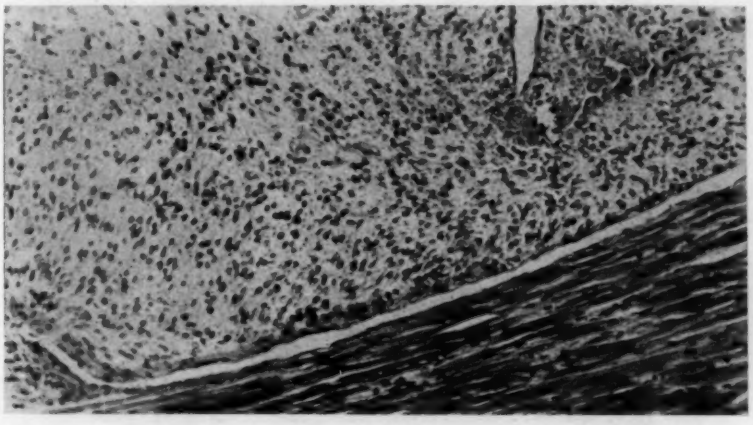

Fig. 2 (case 1).—Section of the tumor and adjacent heart muscle. Hematoxylin and eosin stain; × 120.

Microscopic Examination.—The mediastinal mass was unusual, with a somewhat varying picture in different parts. The striking feature was the areas made up of ribbon-like bands of cells, branching occasionally and showing longitudinal striations. The cytoplasm was acidophilic, and the nuclei were centrally located. The nuclei were oval, with a reticular chromatin network and a very small nucleolus. In some areas the cells became narrow, with fine elongated nuclei, resembling plain muscle. In other parts there was a loose mucoid stroma and the cells were long and spidery but with plump nuclei. There were many thin-walled blood vessels. The tumor of the heart presented a picture resembling that of the mediastinal tumor, with the edges similar to the less differentiated areas of that tumor.

The mediastinal lymph node showed all the features of the mediastinal tumor mass.

The node in the left intercostal muscle disclosed tumor similar to the mediastinal tumor.

Other microscopic alterations were not significant.

Fat stains of the tumor were negative for the presence of fat.

Diagnosis.—Rhabdomyosarcoma of the heart metastasizing to the mediastinal lymph nodes, the pleura and the intercostal muscle.

Case 2.—*Rhabdomyoma of the Heart.*—This tumor was encountered in a 6½ month old stillborn fetus. The mother, 29 years old and white, had the Rh factor in her red cells, group A. She was admitted to the hospital on Nov. 11, 1946 as threatened with abortion. Her last normal menstrual period was stated to have been June 20. She had been regularly attending the prenatal clinic from August 1946 to October 1946. On November 11 she awakened during the night to find herself lying in a pool of blood. She called a doctor, who immediately had her admitted to the hospital. On admission the uterus was 2 fingerbreadths above the umbilicus and there were active fetal movements. On November 12 her temperature was elevated, and penicillin therapy was begun, with resulting improvement and fall of temperature. On November 15 the membranes ruptured spontaneously and the fetus was expelled, followed one hour later by the placenta. The fetus was dead on delivery.

Fig. 3 (case 2).—Cut surface of the heart, showing the tumor attached to the left ventricle. In the wall of the ventricle are several smaller nodules.

After delivery, the patient's illness ran a septic course. She left the hospital against medical advice on November 21.

Gross Examination of the Fetus.—The fetus was female, measuring 24 cm. from crown to rump and 34 cm. from crown to heel, and weighed 820 Gm. The approximate age according to measurements was 26 weeks (Potter and Adair [13]). The skin of the legs was discolored bluish red, and the dorsal surfaces of both feet were edematous. To the umbilicus was attached 10 cm. of umbilical cord, measuring 1.5 cm. in diameter. The heart weighed 7.8 Gm.; the surface vessels were congested, and there were numerous subpericardial petechial hemorrhages.

13. Potter, E. L., and Adair, F. L.: Fetal and Neonatal Death, ed. 1, Chicago, University of Chicago Press, 1940, pp. 23-26.

Located on the left side and attached to the left ventricle was a circumscribed tumor mass, measuring 1.4 by 1.3 by 1.0 cm. The lateral surface was convex, and the medial surface was flattened where it had been applied to the wall of the ventricle. The external surface was gray, and the cut surface was grayish white, smooth and homogeneous. Scattered in the myocardium were several other whitish tumor nodules, measuring as much as 3 mm. in diameter.

The remaining findings were not significant.

Microscopic Examination.—The largest tumor of the myocardium was located beneath the pericardium. Scattered throughout the wall of the left ventricle were numerous well circumscribed tumors. One of these was present in a papillary muscle immediately beneath the endocardium. The tumor cells varied considerably

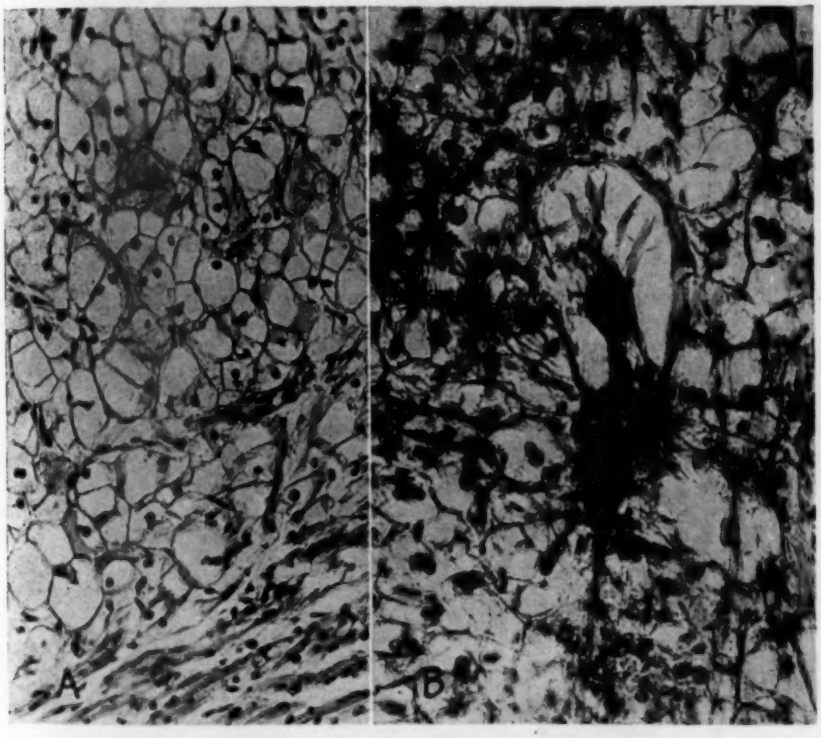

Fig. 4 (case 2).—*A*, photomicrograph of the margin of the largest tumor showing the cytologic structure. Normal myocardium is seen in the lower right corner. Hematoxylin and eosin; × 180. *B*, photomicrograph of a giant spider cell with a centrally located nucleus and radiating cytoplasmic strands separated by vacuoles. Geschickter stain; × 200.

in size, from 10 to 50 microns. The cytoplasm was vacuolated and was granular and netlike, and in areas definite cross striations were seen. The cells did not contain fat. (Staining for glycogen, unfortunately, could not be done, because of incorrect fixation of the tissues.)

Microscopic examination of the remaining fetal parts showed nothing of significance except a large subcapsular hemorrhage of the liver.

The placenta measured 15 by 10 by 3 cm. and weighed 410 Gm. To the fetal surface was attached 25 cm. of umbilical cord. The material surface showed a large part of one pole to be replaced by blood clot which infiltrated throughout the entire thickness of the placenta. The membranes attached to the placenta appeared adequate. Microscopic examination showed the placenta to be well vascularized, with an area of recent hemorrhage.

Diagnosis.—Congenital rhabdomyoma of the heart.

SUMMARY

Two cases of primary tumor of the heart are presented, one a case of rhabdomyosarcoma occurring in a 14 year old boy, the other a case of rhabdomyoma or congenital nodular glycogenic tumor of the heart as an incidental finding in a 26 week fetus.

Notes and News

Appointments, Etc.—A. J. Canny, former senior pathologist and acting director of the Kanematsu Institute of Pathology, Sydney Hospital, has been appointed to the chair of pathology at the University of Queensland, in Australia.

Harry Eagle, of the Johns Hopkins University School of Hygiene and Public Health, has been appointed scientific director of the National Cancer Institute. He will have charge of the extensive program of research within the institute.

Charles S. Cameron has been appointed acting medical and scientific director of the American Cancer Society, following the resignation of Dr. Ashley W. Oughterson.

Hamilton R. Fishback, associate professor of pathology at Northwestern University Medical School and pathologist and director of laboratories at Norwegian-American Hospital, Chicago, has resigned to accept the position of pathologist and director of laboratories at Herrick Memorial Hospital, Berkeley, Calif.

In Brazil, Amadeu Fialho, pathologist of the National Department of Health, has been appointed professor of pathology at the University of Rio de Janeiro, succeeding Paul Leitao da Cunha, who died a few weeks ago.

R. H. Rigdon, formerly professor of pathology at the University of Arkansas School of Medicine has been appointed professor of experimental pathology in the University of Texas, Galveston.

M. D. Eaton, director of the research laboratory of the state department of health at the University of California, has been appointed associate professor of bacteriology and immunology in the Harvard Medical School, Boston.

William D. McNally, toxicologist of the coroner's office of Chicago for twenty-one years, has resigned.

The retirement of S. B. Wolbach as professor of pathology in the Harvard Medical School has been announced. He has accepted the directorship of nutritional research at the Children's Hospital, Boston.

A. C. Ivy, vice president of the University of Illinois, has been appointed executive director of the National Advisory Cancer Council, succeeding George M. Smith, professor emeritus of anatomy in Yale University, who resigned for reasons of health.

Death.—Clement C. Fenton, professor of pathology in the medical school of the University of West Virginia, Morgantown, died May 24, 1947, at the age of 54, from coronary occlusion.

Medicolegal Conference and Seminar.—The departments of legal medicine of the medical schools of Harvard, Tufts and Boston universities, in association with the Massachusetts Medico-Legal Society, will present a six day program (Oct. 13 to 18 1947) of lectures, conferences and demonstrations having to do with the investigation of deaths in the interests of public safety. Attendance will be limited to 25 persons who have registered in advance. Further information may be obtained from the department of Legal Medicine, 25 Shattuck Street, Boston.

Examination for Certification in Pathology.—The American Board of Pathology has declared that after Jan. 1, 1948 all applicants for certification will be required to take the examination of the board. The last meeting of the American Board of Pathology before this date will be from October 24 to 25 in Chicago.

Books Received

BIOCHEMISTRY OF CANCER. By Jesse P. Greenstein, Ph.D., head biochemist and chairman of Section on Biochemistry, National Cancer Institute, National Institute of Health, United States Public Health Service, Bethesda, Md. Cloth. Pp. 389, with 39 illustrations. Price $7.80. New York: Academic Press, Inc., 1947.

The announcement of a new book on the biochemistry of cancer is greeted with enthusiasm in the hope that the extensive recent work in this field will be well summarized and correlated. In a large measure this anticipation is justified. The author is well qualified by a number of years of cancer research to write the book.

The monograph is divided into three sections dealing respectively with the induction of tumors, attempts at control of the induction and the growth of tumors, and the properties of tumors. The first section is subdivided into chapters on extrinsic and intrinsic factors; the second has chapters on nutrition, endocrinologic factors and chemotherapy; the third deals with the chemistry of tumors and of the tumor-bearing host. The monograph thus contains a broader coverage of subject matter than would be expected from the title. On the other hand, little space is given to some chemical subjects, such as immunity.

The third section is the best. It summarizes a large amount of material from many sources. In other sections the presentation of factual material is, on the whole, more impressive than are the generalizations. The important role of chemical studies, as one among many others, in solving problems of the nature and the causation of cancer is repeatedly emphasized. The bibliographies are incomplete but large and useful, nevertheless. While there is no question of accuracy of statements of fact there is sometimes question regarding the best possible shading of emphasis, owing at least in part to lack of inclusiveness which, as the author states, has not been attempted. Despite this lack the book should be useful to all who are concerned with cancer. It is not only an excellent and readable review of the biochemistry of cancer but a good introduction to many other aspects of cancer research. It can be highly recommended to students. Busy pathologists who want a review and reference book should find it valuable.

THE INCIDENCE OF NEUROSIS AMONG FACTORY WORKERS. By Russell Fraser, with the collaboration of Elizabeth Bunbury, Barbara Daniell, M. Elizabeth Barling, F. Estelle Waldron, P. Mary Kemp and Imogen Lee. Medical Research Council, Industrial Health Research Board Report no. 90. Pp. 65. Price 45 cents. London: His Majesty's Stationery Office, 1947.

NEUROPATHOLOGY IN ITS CLINICOPATHOLOGIC ASPECTS. By I. Mark Scheinker, M.D., assistant professor of medicine (neurology) and instructor in neuropathology, University of Cincinnati College of Medicine; neuropathologist and attending neurologist, Cincinnati General Hospital. With a Foreword by Tracy J. Putnam, M.D., professor of neurology and neurological surgery, College of Physicians and Surgeons, Columbia University, and director of services of neurology and neurologic surgery, Neurological Institute of New York. Pp. 306, with 209 illustrations. Price $6.75. Springfield, Ill.: Charles C Thomas, Publisher, 1947.

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